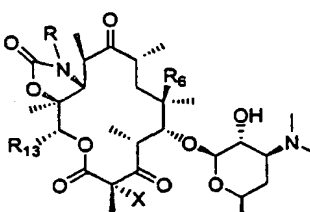




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 31/70, C07H 17/08		A2	(11) International Publication Number: WO 00/62783
			(43) International Publication Date: 26 October 2000 (26.10.00)
(21) International Application Number: PCT/US00/10276		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 13 April 2000 (13.04.00)			
(30) Priority Data: 60/129,729 16 April 1999 (16.04.99) US 06/131,383 28 April 1999 (28.04.99) US 60/140,175 18 June 1999 (18.06.99) US 60/172,159 17 December 1999 (17.12.99) US 60/172,154 17 December 1999 (17.12.99) US			
(71) Applicants: ORTHO-MCNEIL PHARMACEUTICAL, INC. [US/US]; US Route 202, Raritan, NJ 08869 (US). KOSAN BIOSCIENCES, INC. [US/US]; 3832 Bay Center Place, Hayward, CA 94545 (US).		Published <i>Without international search report and to be republished upon receipt of that report.</i>	
(72) Inventors: HLASTA, Dennis; 5008 Davis Drive, Doylestown, PA 18901 (US). HENNINGER, Todd; 125 Bermuda Dr., Neshanic Station, NJ 08853 (US). GRANT, Eugene; 1011 Edison Glen Terrace, Edison, NJ 08837 (US). KHOSLA, Chaitin; 740 Lapara Ave., Palo Alto, CA 94306 (US). CHU, Daniel; 3767 Benton Street, Santa Clara, CA 95051 (US).			
(74) Agents: CIAMPORCERO, Audley et al.; Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, NJ 08933-7003 (US).			
(54) Title: KETOLIDE ANTIBACTERIALS			
			
(57) Abstract <p>A series of ketolide antibacterials in the macrolide family of formula (a), intermediates used in their manufacture and pharmaceutical compositions containing them. The compounds are erythromycin analogues useful in the treatment of bacterial and protozoal infections and in the treatment of other conditions involving gastric motility.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

5

KETOLIDE ANTIBACTERIALS

This application claims priority under 35 USC § 119 from Serial Nos. 60/131,383 filed April 28, 1999, 60/172, 159, filed December 17, 1999, 60/129,729, filed April 16, 1999, 60/172,154 filed December 17, 1999 and 60/140,175 filed June 18, 1999. The contents of these applications are incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to a series of ketolide antibacterials in the macrolide family, intermediates used in their manufacture and pharmaceutical compositions containing them. The compounds are erythromycin analogues useful in the treatment of bacterial and protozoal infections and in the treatment of other conditions involving gastric motility.

20

BACKGROUND OF THE INVENTION

Polyketides are a family of natural products that include many compounds possessing antibiotic and other pharmacologic properties. Erythromycins are a class of macrolide antibiotics originally discovered in 1952 in the metabolic products of a strain of *Streptomyces erythreus*. The antibiotic occurs in various glycosylated forms, designated A, B, C and D. Since their discovery, many have worked to prepare derivatives of the molecule to improve or modify its properties. The focus of much of this work involved chemical modification of the naturally produced erythromycin molecule. This work has produced a number of derivatives including the semi-synthetic antibiotic, clarithromycin, which is 6-O-methylerythromycin. 12,11 oxycarbonyl substituted imino chemical derivatives of erythromycin are described in US patent 5,635,485. However, because of the complexity of the macrolide molecule, medicinal chemistry efforts to produce derivatives have been limited by the kinds of modifications that can be made to the naturally occurring products.

35

Recently, work surrounding the search for modified polyketide antibiotics expanded with the discovery and isolation of the modular polyketide synthases

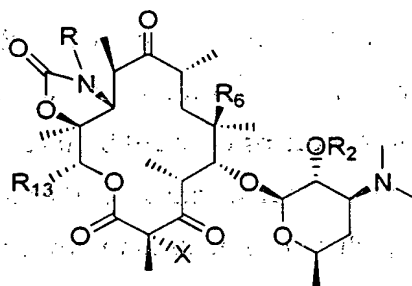
5 (PKS's); multifunctional enzymes related to fatty acid synthases, which catalyze the biosynthesis of polyketides through repeated reactions between acylthioesters to produce the polyketide chain. The entire biosynthetic gene cluster from *S. erythraea* has been mapped and sequenced by Donadio et al. in Industrial Microorganisms: Basic and Applied Molecular Genetics (1993) R.H. Baltz, G.D. Hegeman, and P.L. Skatrud (eds.) (Amer. Soc. Microbiol.) and the entire PKS is a modular assembly of
10 three multifunctional proteins encoded by three separate genes. U.S. Patent 5,672,491 discloses the use of cells transformed with recombinant vectors encoding a variety of PKS gene clusters, which can be used to produce a variety of active polyketides. The vectors can include native or hybrid combinations of PKS subunits, or mutants
15 thereof, to produce a variety of polyketide compounds. Cell-free systems which effect the production of polyketides employing modular polyketide synthases are reported in WO 97/02358.

Using these techniques, production of erythromycin analogues in which C-13
20 bears a substitution other than the natural ethyl group have been reported, for example in WO 98/01571, WO 99/35157, WO 99/03986, and WO 97/02358. WO 98/0156 describes a hybrid modular PKS gene for varying the nature of the starter and extender units to synthesize novel polyketides, including erythromycin analogues. U. S. Patent Nos. 5,824,513 and 6,004,787 further describe methods to produce
25 polyketide structures by introducing specific genetic alterations to genes encoding PKS in the EryA sequence of *S. erythraea*.

The present invention is concerned with novel chemical derivatives of unnatural erythromycin analogues prepared by manipulation of the modular PKS gene
30 clusters.

5

SUMMARY OF THE INVENTION



10

I

This invention is concerned with new compounds of the formula I:

wherein:

15 X is H, F, Cl, Br, or I;

R₂ is selected from H, -COCH₃, and -COPhenyl;

R₆ is selected from H and -O-R₅, where R₅ is substituted or unsubstituted alkyl (C₁-C₁₀), substituted or unsubstituted alkenyl (C₂-C₁₀), or substituted or unsubstituted alkynyl (C₂-C₁₀);

20 R₁₃ is selected from H, (C₁ - C₈) alkyl, 1-alkenyl (C₂-C₈), 1-alkynyl (C₂-C₈), substituted (C₁ - C₈)alkyl, and -CH₂-R" where R" is selected from H, (C₁ - C₈) alkyl, substituted (C₁ - C₈)alkyl, cycloalkyl, alkenyl (C₂- C₈), alkynyl (C₂-C₈), aryl, substituted-aryl, (C₁ - C₈) alkylaryl, heterocyclo, and substituted heterocyclo; provided that R₁₃ can not be ethyl;

25 R is selected from H, aryl, substituted-aryl, heterocyclo, substituted-heterocyclo, cycloalkyl, C₁-C₈-alkyl and C₁-C₈-alkenyl optionally substituted with one or more substituents selected from the group of aryl, substituted-aryl, heterocyclo, substituted-heterocyclo, hydroxy, C₁-C₆-alkoxy;

and the pharmaceutically acceptable salts, esters and pro-drug forms thereof.

30

Listed below are definitions of various terms used to describe this invention. These definitions apply to the terms as they are used throughout this specification,

- 5 unless otherwise limited in specific instances, either individually or as part of a larger group.

The term "alkyl" refers to straight or branched chain unsubstituted hydrocarbon groups of the specified number of carbon atoms.

10

- The term "substituted alkyl" refers to an alkyl group substituted by, for example, one to four substituents, such as, halo, trifluoromethyl, trifluoromethoxy, hydroxy, alkoxy, cycloalkyloxy, heterocylooxy, oxo, alkanoyl, aryloxy, alkanoyloxy, amino, alkylamino, arylamino, aralkylamino, cycloalkylamino, heterocycloamino, disubstituted amines in which the 2 amino substituents are selected from alkyl, aryl or aralkyl, alkanoylamine, aroylamino, aralkanoylamino, substituted alkanoylamino, substituted arylamino, substituted aralkanoylamino, thiol, alkylthio, arylthio, aralkylthio, cycloalkylthio, heterocyclothio, alkylthiono, arylthiono, aralkylthiono, alkylsulfonyl, arylsulfonyl, aralkylsulfonyl, sulfonamido (e.g. SO_2NH_2), substituted sulfonamido, nitro, cyano, carboxy, carbamyl (e.g. CONH_2) substituted carbamyl (e.g. CONH alkyl, CONH aryl, CONH aralkyl or cases where there are two substituents on the nitrogen selected from alkyl, aryl or aralkyl), alkoxycarbonyl, aryl, substituted aryl, guanidino and heterocycles, such as indolyl, imidazolyl, furyl, thienyl, thiazolyl, pyrrolidyl, pyridyl, pyrimidyl and the like. Where noted above where the substituent is further substituted it will be with halogen, alkyl, alkoxy, aryl or aralkyl.

- The term "halogen" or "halo" refers to fluorine, chlorine, bromine and iodine.

- 30 The term "aryl" refers to monocyclic or bicyclic aromatic hydrocarbon groups having 6 to 12 carbon atoms in the ring portion, such as phenyl, naphthyl, and biphenyl groups, each of which may be substituted.

- The term "alkylaryl" or "aralkyl" refers to an aryl group bonded directly through an alkyl group, such as benzyl.

35

5 The term "substituted aryl" refers to an aryl group substituted by, for example, one to four substituents such as alkyl; substituted alkyl, halo, trifluoromethoxy, trifluoromethyl, hydroxy, alkoxy, cycloalkyloxy, heterocycloxy, alkanoyl, alkanoyloxy, amino, alkylamino, aralkylamino, cycloalkylamino, heterocycloamino, dialkylamino, alkanoylamino, thiol, alkylthio, cycloalkylthio, heterocyclothio, ureido, 10 nitro, cyano, carboxy, carboxyalkyl, carbamyl, alkoxycarbonyl, alkylthiono, arylthiono, alkylsulfonyl, sulfonamido, aryloxy and the like. The substituent may be further substituted by halo, hydroxy, alkyl, alkoxy, aryl, substituted aryl, substituted alkyl or aralkyl.

15 The term "cycloalkyl" refers to optionally substituted, saturated cyclic hydrocarbon ring systems, preferably containing 1 to 3 rings and 3 to 7 carbons per ring which may be further fused with an unsaturated C₃-C₇ carbocyclic ring. Exemplary groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl, cyclododecyl, and adamantyl. Exemplary 20 substituents include one or more alkyl groups as described above, or one or more groups described above as alkyl substituents.

 The terms "heterocycle", "heterocyclic" and "heterocyclo" refer to an optionally substituted, fully saturated or unsaturated, aromatic or nonaromatic cyclic 25 group, for example, which is a 4 to 7 membered monocyclic, 7 to 11 membered bicyclic, or 10 to 15 membered tricyclic ring system, which has at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2 or 3 heteroatoms selected from nitrogen atoms, oxygen atoms and sulfur atoms, where the nitrogen and sulfur heteroatoms 30 may also optionally be oxidized and the nitrogen heteroatoms may also optionally be quaternized. The heterocyclic group may be attached at any heteroatom or carbon atom.

 Exemplary monocyclic heterocyclic groups include pyrrolidinyl, pyrrolyl, 35 indolyl, pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazolinyl, imidazolidinyl, oxazolyl, oxazolidinyl, isoxazolinyl, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl,

- 5 piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, 2-oxazepinyl, azepinyl, 4-piperidonyl, pyridinyl, N-oxo-pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, tetrahydropyranyl, tetrahydrothiopyranyl, tetrahydrothiopyranyl sulfone, morpholinyl, thiomorpholinyl, thiomorpholinyl sulfoxide, thiomorpholinyl sulfone, 1,3-dioxolane and tetrahydro-1, 1-dioxothienyl, dioxanyl, isothiazolidinyl, thietanyl, thiiranyl, triazinyl, and triazolyl, and the like. Preferred heterocyclo groups include pyridinyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isoxazolyl, thiadiazolyl, oxadiazolyl, thienyl, furanyl, quinolinyl, isoquinolinyl, and the like.

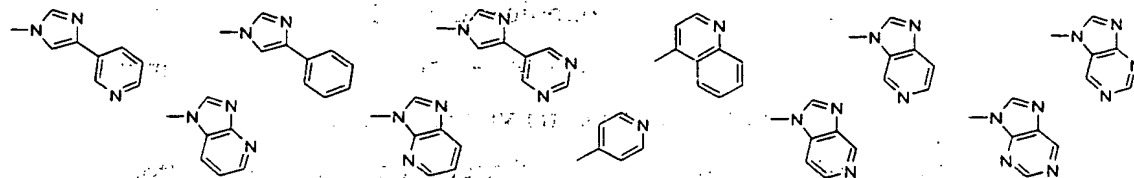
- Exemplary bicyclic heterocyclic groups include benzothiazolyl, benzoxazolyl, benzothienyl, quinuclidinyl, quinolinyl, quinolinyl-N-oxide, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuryl, chromonyl, coumarinyl, cinnolinyl, quinoxalyl, indazolyl, pyrrolopyridinyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,2-b]pyridinyl), or furo[2,3-b]pyridinyl), imidazopyridinyl (such as imidazo[4,5-b]pyridinyl or imidazo[4,5-c]pyridinyl), dihydroisoindolyl, dihydroquinazolyl (such as 3,4-dihydro-4-oxo-quinazolyl), benzisothiazolyl, benzisoxazolyl, benzodiazinyl, benzofurazanyl, benzothiopyranyl, benzotriazolyl, benzpyrazolyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, dihydrobenzopyranyl, indolinyl, isochromanyl, isoindolinyl, naphthyridinyl, phthalazinyl, piperonyl, purinyl, pyridopyridyl, quinazolyl, tetrahydroquinolinyl, thienofuryl, thienopyridyl, thienothienyl, and the like.

- "Substituted heterocyclo" refers to heterocycle substituted by one to four substituents. Exemplary substituents include one or more alkyl groups as described above or one or more groups described above as alkyl substituents. Substituted-heterocyclo may be substituted with a mono-oxo to give for example a 4-oxo-1H-quinoline. Substituted-heterocyclo may also be substituted with a substituted-aryl or a second substituted-heterocyclo to give for example a 4-phenylimidazol-1-yl or a 4-(pyridin-3-yl)-imidazol-1-yl.

35

The following substituted heterocyclo groups are particularly preferred

5



10

The compounds of the invention are those of formula I as set forth above, as well as any stereoisomeric forms of these compounds as shown. The particular stereoisomers depicted are those resulting from the preferred method of synthesis set forth above and exemplified herein; however, by modifying the expression system for the PKS, or by altering the chirality of the diketide, or by synthetic chemical

15 conversion, other stereoisomers may also be prepared. Additional chiral centers may be present in the substituents, such as R_a and R_{13} . The stereoisomers may be administered as mixtures, or individual stereoisomers may be separated and utilized as is known in the art.

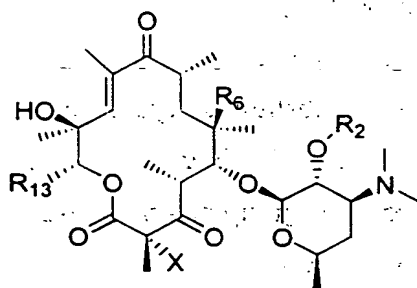
20

The compounds are expected to possess antibacterial activity against Gram positive, Gram negative, and anaerobic bacteria due to their novel structure, and are expected to be useful as broad spectrum antibacterial agents for the treatment of bacterial infections in humans and animals. These compounds are particularly expected to have antimicrobial activity against *S. aureus*, *S. epidermidis*, *S.*

25 *pneumoniae*, *S. pyogenes*, enterococci, *Moraxella catarrhalis* and *H. influenzae*. These compounds are particularly expected to be useful in the treatment of community-acquired pneumonia, upper and lower respiratory tract infections, skin and soft tissue infections, meningitis, hospital-acquired lung infections, and bone and joint infections.

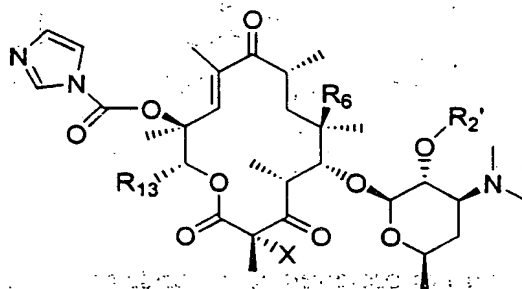
30

Also included in the present invention are compounds useful as intermediates for producing the compounds of the present invention. Such intermediate compounds include those of the formula:



2

Wherein X, R₆ and R₁₃ are as described above, and R₂ is hydrogen or a hydroxyl protecting group, such as acetyl, benzoyl, or trimethylsilyl. Other intermediates included within the scope of this invention are those of the formula:



3

Wherein X, R₆ and R₁₃ are as described above, and R₂ is hydrogen or a hydroxyl protecting group, such as acetyl, benzoyl, or trimethylsilyl.

DETAILED DESCRIPTION

Preferred compounds are those of Formula I wherein:

X is H or F;

R₂ is as described above,

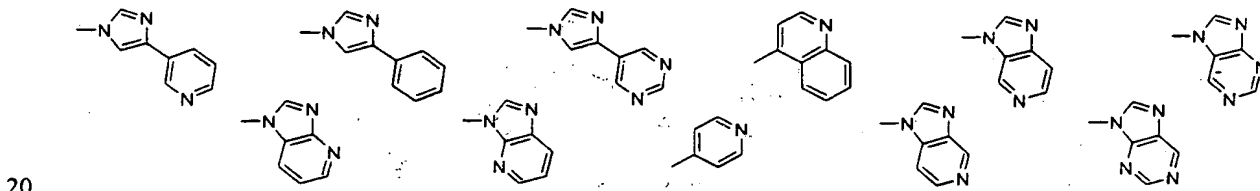
R₆ is selected from H and (C₁ - C₈) alkoxy;

R₁₃ is selected from H, (C₁ - C₈) alkyl, 1-alkenyl (C₂-C₈) (particularly vinyl), 1-

alkynyl (C₂-C₈), haloalkyl, and -CH₂-R" where R" is selected from H, (C₁ - C₈) alkyl,

- 5 haloalkyl, 1-alkenyl (C_2-C_8), 1-alkynyl (C_2-C_8), phenyl, and (C_1-C_8) alkylphenyl (such as benzyl and phenethyl);
 provided that R_{13} can not be ethyl;
 R is selected from H, aryl, substituted-aryl, heterocyclo, substituted-heterocyclo, cycloalkyl, C_1-C_8 -alkyl and C_1-C_8 -alkenyl optionally substituted with one or more
 10 substituents selected from the group of aryl, substituted-aryl, heterocyclo, substituted-heterocyclo, hydroxy, C_1-C_6 -alkoxy;
 and the pharmaceutically acceptable salts, esters and pro-drug forms thereof.

- Particularly preferred groups for R include H, phenyl, C_1-C_8 -alkyl or C_1-C_8 -alkenyl optionally substituted with one or more substituents selected from the group
 15 of phenyl, hydroxy, and the following substituted heterocyclo groups.



Another group of preferred compounds are those of the formula I wherein:

- X is H or F;
 R_2 is as described above,
 25 R_6 is $O-R_a$ where R_a is substituted or unsubstituted alkyl (C_1-C_{10}), substituted or unsubstituted alkenyl (C_2-C_{10}), or substituted or unsubstituted alkynyl (C_2-C_{10});
 R_{13} is selected from H, (C_1-C_8) alkyl, 1-alkenyl (C_2-C_8) (particularly vinyl), 1-alkynyl (C_2-C_8), haloalkyl, and $-CH_2-R''$ where R'' is selected from H, (C_1-C_8) alkyl, haloalkyl, 1-alkenyl (C_2-C_8), 1-alkynyl (C_2-C_8), phenyl, and (C_1-C_8) alkylphenyl
 30 (such as benzyl and phenethyl);
 provided that R_{13} can not be ethyl;
 R is H;
 and the pharmaceutically acceptable salts, esters and pro-drug forms thereof.

- 35 Particularly preferred groups for R_a include optionally substituted 3-(quinolin-3-yl)prop-2-enyl, optionally substituted 3-(quinolin-3-yl)prop-2-ynyl, optionally substituted 3-(quinolin-6-yl)prop-2-enyl, optionally substituted 3-(quinolin-6-yl)prop-

5 2-ynyl, optionally substituted 3-(quinolin-7-yl)prop-2-enyl, optionally substituted 3-phenylprop-2-enyl, optionally substituted 3-(naphth-1-yl)prop-2-enyl, optionally substituted 3-(naphth-1-yl)prop-2-ynyl, optionally substituted 3-(naphth-2-yl)prop-2-enyl, optionally substituted 3-(naphth-2-yl)prop-2-ynyl, optionally substituted 5-phenylpent-4-en-2-ynyl, optionally substituted 3-(fur-2-yl)prop-2-ynyl, optionally substituted 3-(thien-2-yl)prop-2-enyl, optionally substituted 3-(carbazol-3-yl)prop-2-enyl, and optionally substituted 3-(quinoxalin-6-yl)prop-2-enyl.

The preferred compounds of this invention may be prepared according to the following reaction schemes:

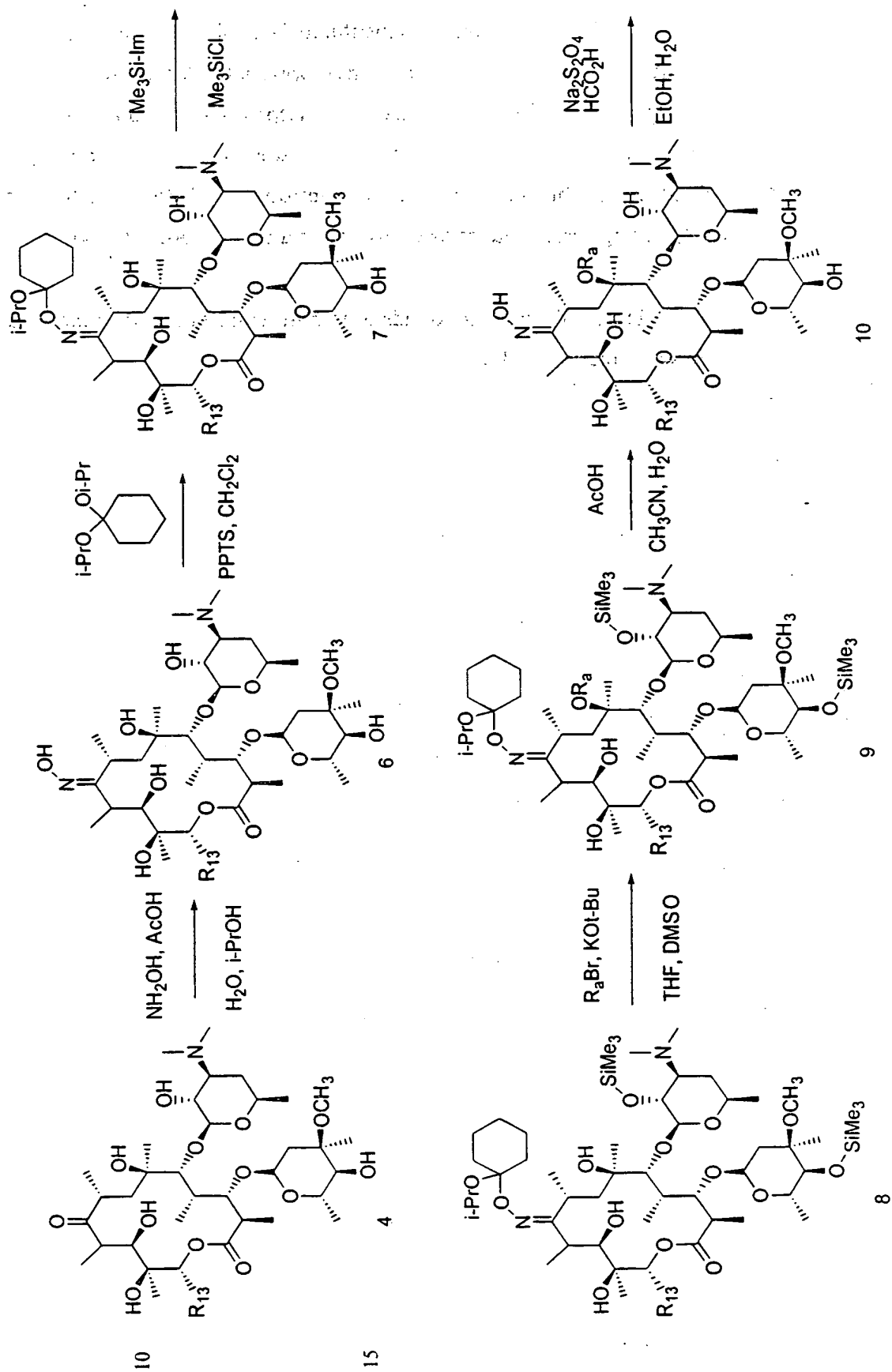
15

20

25

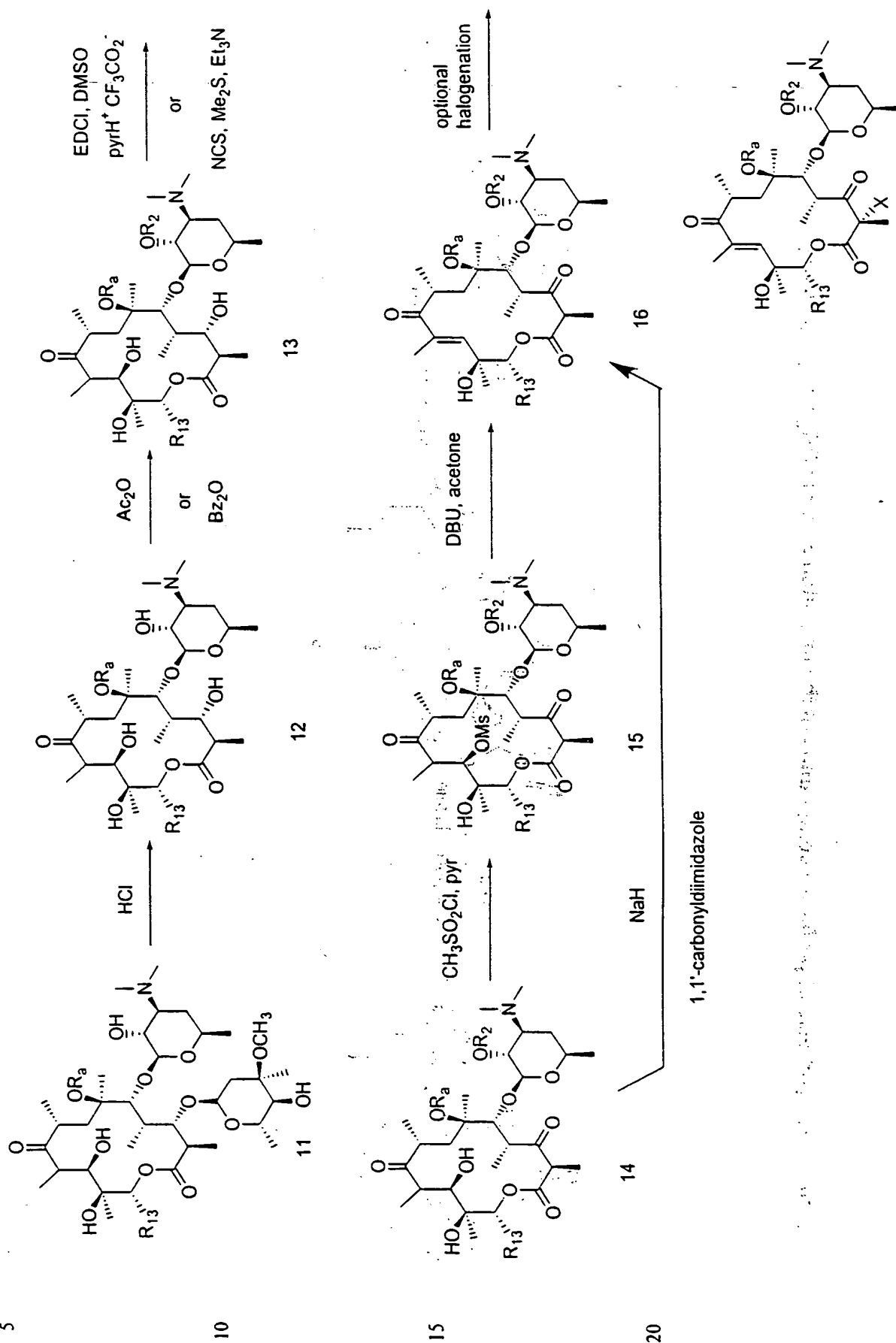
30

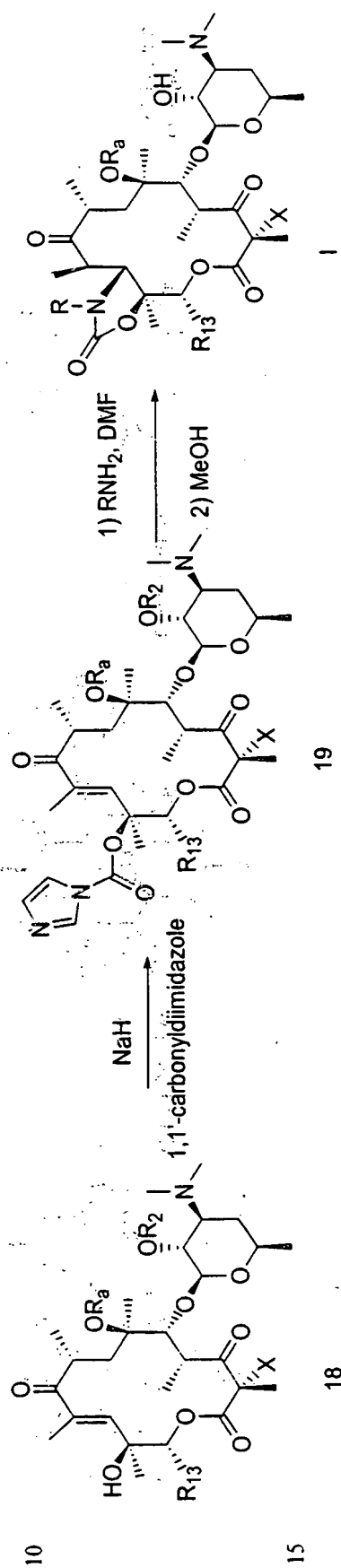
Scheme 1



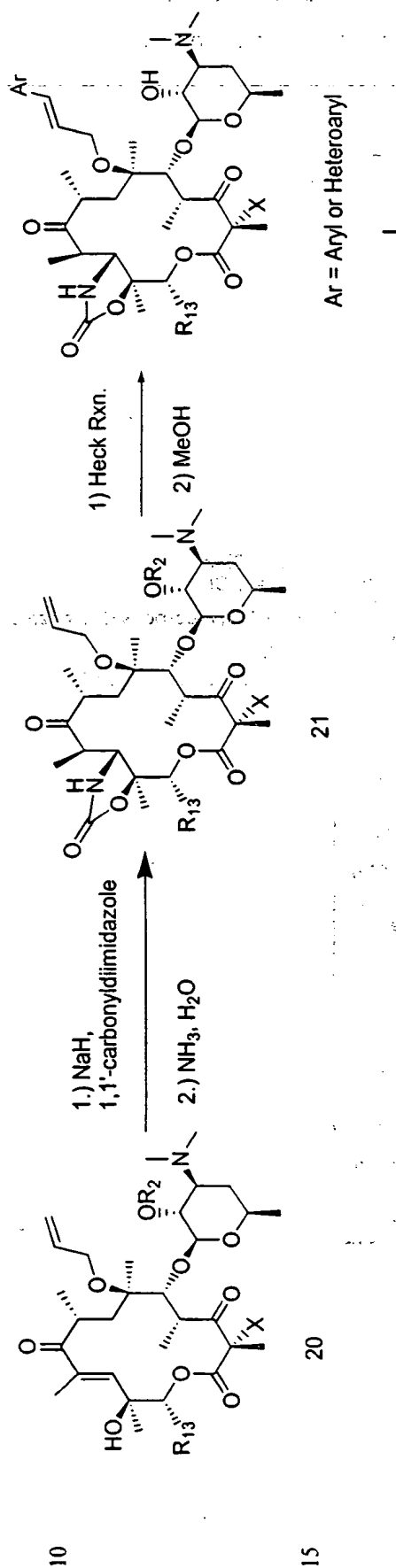
- 12 -

Scheme 1 (cont.)



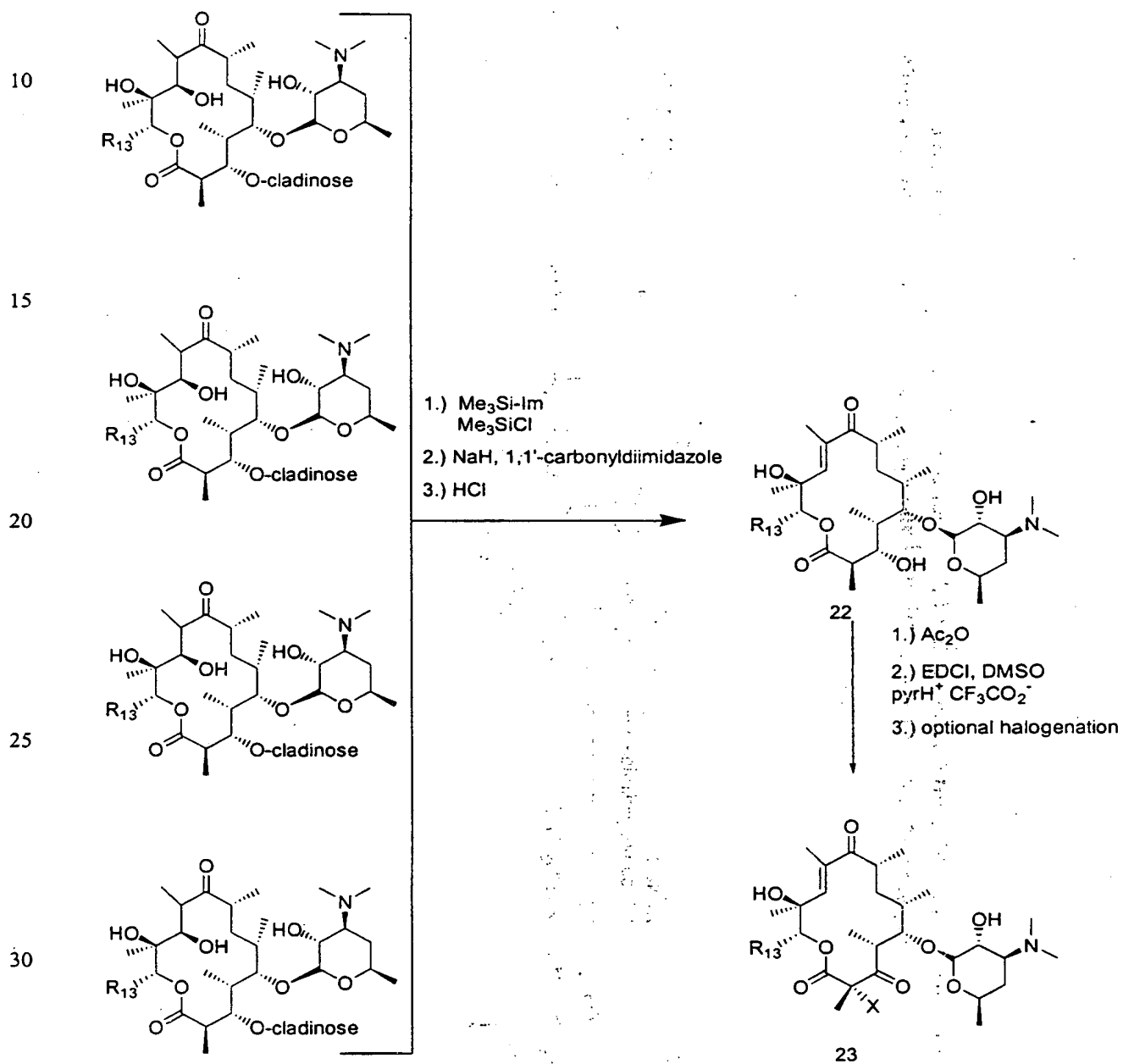
Scheme 2: Synthesis of preferred compounds of the invention wherein $OR_a = (C_1-C_8)$ alkoxy

Scheme 3: Synthesis of the preferred compounds of the invention wherein R_a = substituted or unsubstituted alkyl (C_1 - C_{10}), substituted or unsubstituted alkenyl (C_2 - C_{10}), or substituted or unsubstituted alkynyl (C_2 - C_{10})

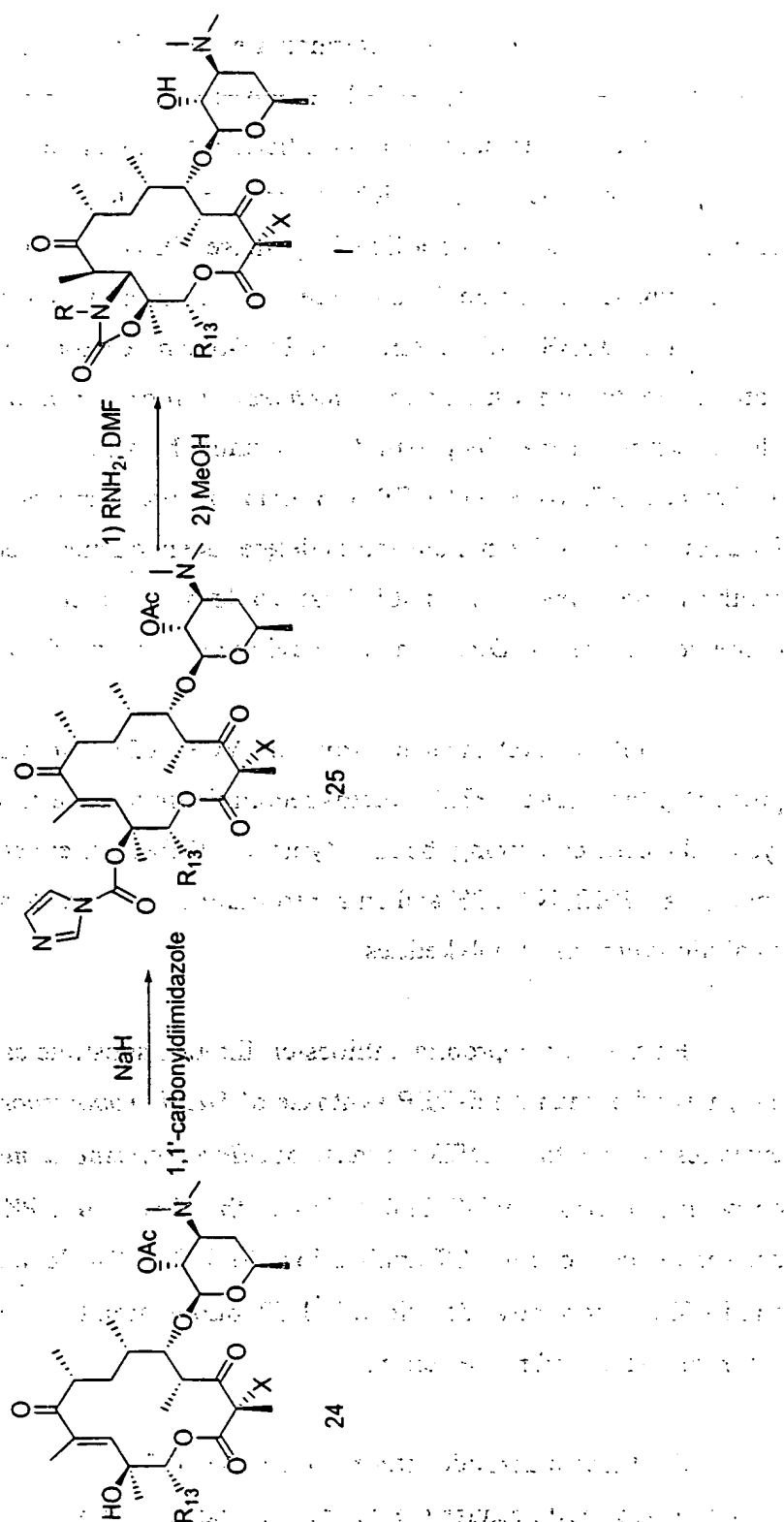


5 The preferred compounds of the invention wherein R_6 is hydrogen may be prepared according to the following reaction scheme:

Scheme 4



Scheme 4 (cont.)



ORT 979

5

The starting macrolide intermediates, 4 and 5, may be prepared by methods described in U.S. Patent 5,672,491, hereby incorporated by reference into the present application. In one embodiment, modified erythromycin intermediates, 4 and 5, may be prepared by a method in which an appropriate thioester diketide substrate is provided to 6-deoxyerythronolide B synthase (DEBS) that is unable to act on its natural substrate (propionyl CoA) due to a mutation in the ketosynthase domain of module 1 of DEBS. This recombinant DEBS can be expressed in the organism that natively produces erythromycin, *Saccharopolyspora erythraea*, or the entire gene cluster can be inserted by plasmid in a suitable host such as *S. coelicolor* (Jacobsen et al, Science 277:367-369 (1997)) or *S. lividans*, preferably an *S. coelicolor* or *S. lividans* which has been modified to delete its endogenous actinorhodin polyketide synthesis mechanism. A suitable host would be *S. coelicolor* CH999/pJRJ2, which expresses a mutant 6-DEB synthase having an inactivated module 1 ketosynthase.

20

A cell free system as described in WO97/02358 may also be employed by producing the relevant PKS proteins recombinantly and effecting their secretion or lysing the cells containing them. A typical cell-free system would include the appropriate PKS, NADPH and an appropriate buffer and substrates required for the catalytic synthesis of polyketides.

25

Further, the appropriate thioester diketide substrate can be provided to PKS enzymes other than the 6-DEB synthase of *Saccharopolyspora erythraea*. Other PKS enzymes include the 6-DEB synthase of *Micromonospora megalomicea* and its KS1° derivative described in USSN 60/158305, the oleandolide PKS and its KS1° derivative described in PCT application No. US 99/24478, and the narbonolide PKS and its KS1° derivative described in PCT publication No. WO 99/61599, all incorporated by reference herein.

30

For those macrolide intermediates, 4 and 5, where R₁₃ is methyl, no diketide feeding is required because the desired aglycone may be produced by the recombinant host cell *Streptomyces coelicolor* CH999/pCK7, as further described herein.

35

5 The resulting aglycones thus prepared are then added to the fermentation broth of *Saccharopolyspora erythraea* strains which chemically glycosylate at the 3 and 5 positions, hydroxylate at C-12, and optionally hydroxylate at the 6 position, depending on the strain employed.

10 The modified erythromycins of the invention, in addition to modification at C-13, contain an -OH group at position 6 unless -OH is replaced by H or OR_a as described above. To construct, ultimately, the compounds of formula I where position 6 is OR_a, the compound of formula (4) is provided with protecting groups on the hydroxyl groups of the two glucose residues (Scheme 1). Such protection is effected
15 using suitable protecting reagents such as acetic anhydride, benzoic anhydride, benzyl chloroformate, hexamethyldisilazane, or a trialkylsilyl chloride in an aprotic solvent. Aprotic solvents include, for example, dichloromethane, chloroform, tetrahydrofuran, N-methyl pyrrolidone, dimethyl sulfoxide (DMSO), dimethyl formamide (DMF) and the like. Mixtures may also be used. Protection of both sugar hydroxyls in formula
20 (4) may be done simultaneously or sequentially.

 In addition to protecting the 2' and 4'' hydroxyl groups of the two glucose residues, the keto group at position 9 of the macrolide ring must also be protected. Typically, this is effected by converting the keto group to a derivatized oxime.
25 Particularly preferred embodiments for R in the formula =NOR include unsubstituted or substituted alkyl (1-12C), substituted or unsubstituted aryl (6-10C), alkyl (1-12C), substituted or unsubstituted heteroaryl (6-10C), alkyl (1-12C), and heteroalkyl (such as substituents of the formula CR'₂OR wherein each R', in addition to being independently embodied as R as set forth above, may, together with the other, form a
30 cycloalkyl ring (3-12C)). A preferred derivatized oxime is of the formula =NOR wherein R is isopropoxycyclohexyl.

 With the 9-keto group and the 2' and 4'' hydroxyls protected, it is then possible to alkylate the 6-hydroxy group in the compound of formula (8) by reaction
35 with an alkylating agent in the presence of base. Alkylating agents include alkyl halides and sulfonates. For example, the alkylating agents may include methyl tosylate, 2-fluoroethyl bromide, cinnamyl bromide, crotonyl bromide, allyl bromide,

5 propargyl bromide, and the like. The alkylation is conducted in the presence of base, such as potassium hydroxide, sodium hydride, potassium isopropoxide, potassium t-butoxide, and an aprotic solvent.

The choice of alkylating agent will depend on the nature of the substituents R_1 to be included. As set forth above, R_1 can be substituted or unsubstituted alkyl (1-10C), substituted or unsubstituted alkenyl (2-10C), or substituted or unsubstituted alkynyl (2-10C). Particularly preferred are unsubstituted alkyl, alkenyl, or alkynyl, or substituted forms of these wherein the substituents include one or more halogen, hydroxy, alkoxy (1-6C), oxo, SO_2R (1-6C), N_3 , CN, and NR_2 , wherein R is H, substituted or unsubstituted alkyl (including cycloalkyl) (1-12C), substituted or unsubstituted alkenyl (including cycloalkenyl) (2-12C), alkynyl (including cycloalkynyl) (2-12C), substituted or unsubstituted aryl (6-10C), including the hetero forms of the above.

20 Especially preferred are methyl, allyl and ethyl.

Once the alkylation of the 6-hydroxyl is completed, the sugar residues and the macrolide ring may be deprotected. Deprotection of the glycoside moieties is conducted as described by Green, T.W., *et al.*, in Protective Groups in Organic
25 Synthesis, *infra*. Similar conditions result in converting the derivatized oxime to =NOH. If formation of the underivatized oxime is not concurrent with deprotection, the conversion to the oxime is conducted separately.

The oxime can then be removed and converted to a keto group by standard
30 methods known in the art. Deoximating agents include inorganic sulfur oxide compounds such as sodium hydrogen sulfite, sodium pyrosulfate, sodium thiosulfate, and the like. In this case, protic solvents are used, such as water, methanol, ethanol, isopropanol, trimethyl silanol and mixtures of these. In general, the deoximation reaction is conducted in the presence of an organic acid.

35 At this point in the process, or later, the group introduced at the 6-hydroxyl can further be manipulated. Conveniently, the initial substitution may provide a

5 6-O-allyl, i.e., $\text{O-CH}_2\text{CH=CH}_2$, which can further be derivatized by reduction to give the 6-O propyl compound, or be treated with osmium tetroxide to provide the 2,3-dihydroxypropyl compound, which can further be esterified at each oxygen atom. The O-allyl derivative can also be oxidized with m-chloroperoxybenzoic acid in an aprotic solvent to provide the epoxy compound which can be opened with amines or
10 N-containing heteroaryl compounds to provide compounds with N-containing side-chains, or can be oxidized under Wacker conditions to provide the substituent $\text{O-CH}_2\text{-C(O)-CH}_3$, or can be ozonized to provide the aldehyde. The aldehyde can then be converted to the oxime or reacted with a suitable amine and reduced in the presence of a borohydride reducing agent to provide an amine. The oxime can also be converted
15 to a nitrile by reaction with a dehydration agent in an aprotic solvent. The O-allyl derivative can also be reacted with an aryl halide under Heck conditions (Pd(II) or Pd(O), phosphine and amine or inorganic base) to provide a 3-aryl prop-2-enyl derivative (Scheme 3). This derivative can then be reduced with hydrogen and palladium on carbon to provide a 3-arylpropyl derivative. If the initial substituent R_a
20 is a 2-propyne, similar reactions can be employed to provide alterations in the side-chain, including arylation.

In order to convert the compound of formula (11) into the compound of formula (12), by first removing the cladinose moiety, the compound of formula (11) is
25 treated with mild aqueous acid or with a deglycosylating enzyme (Scheme 1). Suitable acids include hydrochloric, sulfuric, chloroacetic, trifluoroacetic and the like, in the presence of alcohol. Reaction times are typically 0.5-24 hours at a temperature of -10 - 35°C . Following protection of the 2' hydroxyl of the remaining sugar as set forth above, the resulting hydroxyl group at the 3-position of the macrolide ring is
30 then oxidized to the ketone using a modified Swern oxidation procedure. In this procedure, an oxidizing agent such as N-chlorosuccinimide-dimethyl sulfide or a carbodiimide-dimethylsulfoxide is used. Typically, a compound of formula (13) is added to a pre-formed N-chlorosuccinimide and dimethyl sulfide complex in a chlorinated solvent such as methylene chloride at -10 - 25°C . After being stirred for
35 0.5-4 hours, a tertiary amine such as triethylamine is added to produce the corresponding ketone.

5 Intermediate (16) can then be prepared from intermediate (14) in a two-step procedure as illustrated in Scheme 1. First the 11-hydroxyl group is preferentially converted to a leaving group, such as methanesulfonate, by reaction with an alkyl or arylsulfonyl chloride, such as methanesulfonyl chloride, in the presence of an organic base, like pyridine. In the next step, the leaving group is eliminated to afford the 10-11 double bond by treatment with diazabicycloundecane in a suitable solvent like acetone.

In order to halogenate the macrolide at position 2 (converting X=H to halogen), the compound of formula (16), is treated with a base and an electrophilic halogenating reagent such as pyridinium perbromide or N-fluorobenzene sulfonic acid.

In accordance with Scheme 2, intermediate (18) is reacted with sodium hydride and 1,1'-carbonyldiimidazole to yield the imidazole intermediate (19). Intermediate (19) is then reacted with the appropriate substituted amine in dimethylformamide at about 25° to 130° and then the hydroxy protecting group is removed by treatment with methanol to yield the final compound I.

In a similar fashion (Scheme 3), intermediate (20) can be converted to the unsubstituted 11,12-cyclic carbamate intermediate (21) by reaction of the analogous imidazole intermediate with aqueous ammonia. As mentioned earlier, the 6-position substituent can be manipulated late in the synthetic sequence, and this is illustrated in Scheme 3, where the 6-O-allyl derivative is reacted with an aryl halide under Heck conditions (Pd(II) or Pd(O), phosphine and amine or inorganic base) to provide a 3-aryl prop-2-enyl derivative. Subsequent deprotection of the 2' hydroxyl group affords the final compound I.

It will also be noted that compounds of the invention wherein R₆ is hydrogen can be readily obtained from a mixture of 6-deoxy-13-substituted-erythromycins A, B, C, and D derived from fermentation. A similar series of reactions as described above can be used to effect this conversion (Scheme 4).

5 This invention further provides a method of treating bacterial infections, or enhancing the activity of other antibacterial agents, in warm-blooded animals, which comprises administering to the animals a compound of the invention alone or in admixture with a diluent or in the form of a medicament according to the invention.

10 When the compounds are employed for the above utility, they may be combined with one or more pharmaceutically acceptable carriers, e.g., solvents, diluents, and the like, and may be administered orally in such forms as tablets, capsules, dispersible powders, granules, or suspensions containing for example, from about 0.5% to 5% of suspending agent, syrups containing, for example, from about 10% to 50% of sugar,
15 and elixirs containing, for example, from about 20% to 50% ethanol, and the like, or parenterally in the form of sterile injectable solutions or suspensions containing from about 0.5% to 5% suspending agent in an isotonic medium. These pharmaceutical preparations may contain, for example, from about 0.5% up to about 90% of the active ingredient in combination with the carrier, more usually between 5% and 60%
20 by weight.

 Compositions for topical application may take the form of liquids, creams or gels, containing a therapeutically effective concentration of a compound of the invention admixed with a dermatologically acceptable carrier.

25 In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed. Solid carriers include starch, lactose, dicalcium phosphate, microcrystalline cellulose, sucrose and kaolin, while liquid carriers include sterile water, polyethylene glycols, non-ionic surfactants and edible
30 oils such as corn, peanut and sesame oils, as are appropriate to the nature of the active ingredient and the particular form of administration desired. Adjuvants customarily employed in the preparation of pharmaceutical compositions may be advantageously included, such as flavoring agents, coloring agents, preserving agents, and antioxidants, for example, vitamin E, ascorbic acid, BHT and BHA.

35 The preferred pharmaceutical compositions from the standpoint of ease of preparation and administration are solid compositions, particularly tablets and hard-filled or liquid-filled capsules. Oral administration of the compounds is preferred.

40 These active compounds may also be administered parenterally or intraperitoneally. Solutions or suspensions of these active compounds as a free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a

5 surfactant such as hydroxypropyl-cellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

10 The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the
15 contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

20 The effective dosage of active ingredient employed may vary depending on the particular compound employed, the mode of administration and the severity of the condition being treated. However, in general, satisfactory results are obtained when the compounds of the invention are administered at a daily dosage of from about 0.1 mg/kg to about 400 mg/kg of animal body weight, preferably given once a day, or in
25 divided doses two to four times a day, or in sustained release form. For most large mammals the total daily dosage is from about 0.07 g to 7.0 g, preferably from about 100 mg to 1000 mg. Dosage forms suitable for internal use comprise from about 100 mg to 500 mg of the active compound in intimate admixture with a solid or liquid pharmaceutically acceptable carrier. This dosage regimen may be adjusted to provide
30 the optimal therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The production of the above-mentioned pharmaceutical compositions and
35 medicaments is carried out by any method known in the art, for example, by mixing the active ingredient(s) with the diluent(s) to form a pharmaceutical composition (e.g. a granulate) and then forming the composition into the medicament (e.g. tablets).

Examples

40 The compounds of the invention can be prepared using intermediates produced by a chemobiosynthetic procedure involving recombinant host cells and organic

5 chemistry methodology. The steps of this chemobiosynthetic procedure are described generally below, followed by a detailed description of each step in the enumerated Examples.

In the first general step of the method, a 6-deoxyerythronolide B (6-dEB)
10 derivative compound is prepared by fermentation of a recombinant *Streptomyces* host cell.

The fermentation to produce 15-methyl-6-deoxyerythronolide B and 14,15-dehydro-6-deoxyerythronolide B requires a synthetic diketide intermediate to be fed
15 to the fermenting cells. The preparation of these synthetic diketides is described in Example 1. These synthetic diketides are substrates for a 6-deoxyerythronolide B synthase (DEBS) that is unable to act on its natural substrate (propionyl CoA) due to a mutation in the ketosynthase domain of module 1 of DEBS. This recombinant DEBS is provided by plasmid pJRJ2 in *Streptomyces coelicolor* CH999. *S. coelicolor* CH999
20 is described in U.S. Patent No. 5,672,491, incorporated herein by reference. A derivative of *S. coelicolor* CH999, *S. coelicolor* K39-02, that has been genetically modified to include a *ptpA* gene, is described in U.S. patent application Serial No. 09/181,833, incorporated herein by reference can also be employed for this purpose. Plasmid pJRJ2 encodes the *eryAI*, *eryAII*, and *eryAIII* genes; the *eryAI* gene
25 contained in the plasmid contains the KS1 null mutation. The KS1 null mutation prevents formation of the 6-deoxyerythronolide B produced by the wild-type gene unless exogenous substrate is provided. Plasmid pJRJ2 and a process for using the plasmid to prepare novel 13-substituted erythromycins are described in PCT publication Nos. 99/03986 and 97/02358 and in U.S. patent application Serial Nos.
30 08/675,817, filed 5 July 1996; 08/896,323, filed 17 July 1997; and 09/311,756, filed 14 May 1999, each of which is incorporated herein by reference. The exogenous substrates provided can be prepared by the methods and include the compounds described in PCT patent application No. PCT/US00/02397 and U.S. patent application Serial No. 09/492,733, both filed 27 Jan. 2000, by inventors G. Ashley et al., and both
35 of which claim priority to U.S. patent application Serial No. 60/117,384, filed 27 Jan. 1999, each of which is incorporated herein by reference. PKS genes other than the *ery* genes can also be employed; suitable genes include the KS1 null mutation containing

5 oleandolide and megalomicin PKS genes described in U.S. patent application Serial Nos. 60/158,305, filed 8 Oct. 1999 and 09/428,517, filed 28 Oct. 1999, and PCT application No. US99/24478, filed 22 Oct. 1999, each of which is incorporated herein by reference.

10 The fermentation to produce 14-nor-6-deoxyerythronolide B does not require diketide feeding, because the desired compound is produced by the recombinant host cell *Streptomyces coelicolor* CH999/pCK7. Plasmid pCK7 is described in U.S. Patent No. 5,672,491 and comprises the DEBS genes. A derivative of plasmid pCK7, pKOS011-26, can also be used. The host cell comprising pKOS011-26 and a
15 recombinant ptpA gene is called *S. coelicolor* 27-26/pKOS011-26. These host cells produce both 6-deoxyerythronolide B and 14-nor-6-deoxyerythronolide, due to the incorporation of propionyl CoA and acetyl CoA, both of which serve as substrates for DEBS.

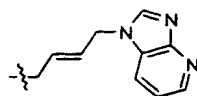
20 The fermentation of *Streptomyces coelicolor* CH999/pJRJ2 and *S. coelicolor* CH999/pCK7 is described in Example 2. The isolation of the 6-deoxyerythronolide products resulting from this fermentation is also described in Example 2.

 The isolated products are then added to the fermentation broth of *Saccharopolyspora erythraea* strains to make other useful intermediate compounds of
25 the invention. The *S. erythraea* strains catalyze the biosynthesis and attachment of sugar residues to the 3 and 5 positions of the 6-dEB derivative compounds. These strains also comprise a functional eryK gene product and so hydroxylate the 6-dEB derivative compounds at the 12 position. The strains differ in regard to whether a functional eryF gene product is produced. If so, then the compounds produced are
30 hydroxylated at the 6 position as well. If not, then a 6-deoxyerythromycin A derivative is produced. These *S. erythraea* fermentations are described in Example 3, together with the isolation of the erythromycin A derivative compounds from the fermentation broth.

35 The isolated products are then used as intermediates in the chemical synthesis of other intermediate compounds of the invention. For erythromycin A derivative compounds of the invention that comprise a 6-hydroxyl, Examples 4 – 6, 11, and 16

5 describe the process for alkylating the compounds to make the 6-O-alkyl, 6-O-allyl, and 6-O-propargyl intermediates of the invention, as depicted in Scheme 1 [Intermediates (8) -> (11)].

For erythromycin A derivative compounds of the invention that comprise the 6-O-alkyl groups, Examples 7-9 describe the process for making the 10,11- anhydro compounds of the invention. This reaction sequence is depicted in Scheme 1 [Intermediates (11) -> (16)]. Example 10 describes the process for making the 2-halo compounds of the invention. In particular, the compound of formula (26), is treated with a base and an electrophilic halogenating reagent such as pyridinium perbromide or N-fluorobenzenesulfonic acid, as depicted in Scheme 5. Example 12 describes the process for removing the cladinose sugar from erythromycin A derivatives containing the 6-O-allyl group and for oxidation of the resulting 3-hydroxyl group to the ketone [Scheme 1; Intermediates (11) -> (14)]. Example 13 illustrates the conversion of the compounds containing the 6-O-allyl group to several useful intermediates in the synthesis of compounds of the invention. Example 14 describes the synthesis of a compound of the invention (I) wherein R = H, R₂ = H, X = H and R₆ = O-allyl [Scheme 1; Intermediates (14) -> (17) and Scheme 3; Intermediate (20) -> (I)]. Example 15 describes the process for conversion of macrolides containing the 6-O-allyl and 11,12-cyclic carbamate functionalities to compounds of the invention (I) via the Heck reaction and subsequent deprotection of the desosamine sugar (Scheme 3; Intermediate (21) -> (I)). In addition to illustrating the alkylation of the compounds to the 6-O-propargyl intermediates, Example 16 further describes the process for conversion of these intermediates to compounds of the invention (I). Example 17 describes the process for conversion of 6-O-propargyl intermediates to another group of compounds of the invention (I) via the Sonogashira reaction. For erythromycin A derivative compounds of the invention that do not comprise a 6-hydroxyl, Examples 18-20 describe the process for making the 10,11- anhydro compounds of the invention. These reaction sequences are depicted in Schemes 4 and 6. Example 21 describes the process for the synthesis of 1H-imidazo[4,5-b]pyridine-1-(4-amino-2-butene), an amine used in the synthesis of compounds of the invention wherein R =



5

This sequence of reactions is depicted in Scheme 7. The process for conversion of the 10,11-anhydro compounds into the carbamate derivative compounds of the invention is described in Examples 22 and 23 [Scheme 3; Intermediate (18) -> (I)]. The amines used in the synthesis of the carbamate derivative compounds of the invention (I) are either commercially available or can be readily prepared as described in Denis et al, Bioorg. Med. Chem. Lett. 9:3075-3080 (1999).

10

Example 1Preparation of Diketide Thioesters

The processes used to prepare the N-acetylcysteaminethioesters (NACS) used to feed the recombinant Streptomyces host cells to make the 15-methyl and 14,15-dehydro-6-deoxyerythronolide B intermediate compounds are described in this Example. The synthesis protocols described below are also described in U.S. provisional patent application Serial No. 09/492,733, inventors G. Ashley, M. Burlingame, and I. Chan-Kai, incorporated herein by reference.

Thus, (2S,3R)-2-methyl-3-hydroxyhexanoate NACS (Preparation E), which is used to prepare the 15-methyl-6-deoxyerythronolide B intermediate, is prepared from reacting (4S)-N-[(2S,3R)-2-methyl-3-hydroxyhexanoyl]-4-benzyl-2-oxazolidinone (Preparation D) with N-acetylcysteamine (Preparation B). N-acetylcysteamine is, in turn, prepared from N,S-diacetylcysteamine (Preparation A). (4S)-N-[(2S,3R)-2-methyl-3-hydroxyhexanoyl]-4-benzyl-2-oxazolidinone (Preparation D) is prepared from (4S)-N-Propionyl-4-benzyl-2-oxazolidinone (Propionyl-Nox; Preparation C).

In similar fashion, (2S,3R)-2-methyl-3-hydroxy-4-pentenoate NACS (Preparation G), which is used to prepare the 14,15-dehydro-6-deoxyerythronolide B intermediate, is prepared from reacting (4S)-N-[(2S,3R)-2-methyl-3-hydroxy-4-pentenoyl]-4-benzyl-2-oxazolidinone (Preparation F) with N-acetylcysteamine (Preparation B). (4S)-N-[(2S,3R)-2-methyl-3-hydroxy-4-pentenoyl]-4-benzyl-2-oxazolidinone (Preparation F) is prepared from (4S)-N-Propionyl-4-benzyl-2-oxazolidinone (Propionyl-Nox; Preparation C).

Preparation A: Preparation of N,S-Diacetylcysteamine

Cysteamine hydrochloride (50.0 g) is added to a 1 L 3-neck round bottom flask fitted with a magnetic stir bar, 2 addition funnels, and a pH electrode. Water (300 mL) is added, and the stirred solution is cooled on ice. The pH is adjusted to 8.0 by addition of 8 N KOH. Acetic anhydride (125 mL) is placed in one addition funnel, and 8N KOH (350 mL) is placed in the other addition funnel. The acetic anhydride is added dropwise to the cysteamine solution, with 8 N KOH being added so as to keep the reaction pH at 8 +/- 1. After addition of acetic anhydride is complete, the pH was adjusted to 7.0 using 1 N HCl and the mixture is allowed to stir for 75 min. on ice. Solid NaCl is added to saturation, and the solution is extracted 4 times using 400 mL

- 5 portions of CH_2Cl_2 . The organic extracts are combined, dried over MgSO_4 , filtered, and concentrated under reduced pressure to yield 68.9 g (97% yield) of a pale yellow oil, which crystallizes upon standing at 4°C .

Preparation B: Preparation of N-Acetylcysteamine

- 10 N,S-diacetylcysteamine (42.64 g) is placed in a 2 L round bottom flask fitted with a magnetic stirrer, and dissolved in 1400 mL of water. The flask is purged with N_2 , and the mixture is chilled in an ice bath. Potassium hydroxide (49.42 g) is added, and the mixture is stirred for 2 hr. on ice under inert atmosphere. The pH is adjusted to 7 using 6 N HCl, and solid NaCl is added to saturation. The mixture is extracted 7
15 times with 500 mL portions of CH_2Cl_2 . The organic extracts are combined, dried over MgSO_4 , filtered, and concentrated under reduced pressure to yield 30.2 g (96% yield) of product. This material is distilled immediately prior to use, bp $138\text{--}140^\circ\text{C}/7\text{ mmHg}$.

Preparation C: Preparation of (4S)-N-Propionyl-4-benzyl-2-oxazolidinone (Propionyl-

- 20 Nox)

- A dry, 1 L three-necked round bottomed flask equipped with a 500 mL addition funnel and a stir bar was charged with 20 g of (4S)-4-benzyl-2-oxazolidinone, capped with septa and flushed with nitrogen. Anhydrous THF (300 mL) was added by cannula and the resulting solution was cooled with a -78°C bath of
25 dry ice/isopropanol. The addition funnel was charged with 78 mL of n-butyllithium (1.6 M in hexane) by cannula, which was added in a slow stream to the reaction. Distilled propionyl chloride (bp $77\text{--}79^\circ\text{C}$), 8.0 mL, was added rapidly via syringe. The reaction was allowed to stir for 30 min. in the dry ice/isopropanol bath.

- The reaction was removed from the cold bath, allowed to warm to $>0^\circ\text{C}$, and
30 quenched with 50 mL of saturated aqueous NH_4Cl . The mixture was concentrated to a slurry on a rotary evaporator. The slurry was extracted three times with 250 mL portions of ethyl ether. The organic extracts were combined and washed with 50 mL each of saturated aqueous NaHCO_3 and brine, dried with MgSO_4 , filtered, and concentrated to give a yellow oil. The material crystallized upon sitting. The crystals
35 were triturated once with cold (-20°C) hexanes to give 21.0 g (80% yield) of white crystalline material, m.p. $41\text{--}43^\circ\text{C}$. APCI-MS: $m/z = 234$ (MH⁺), 178, 117. $^1\text{H-NMR}$ (360 MHz, CDCl_3): 7.2-7.4 (5H,m); 4.67 (1H,m,H4); 4.14-4.22 (2H,m,H5); 3.30

- 5 (1H,dd,J=3,13 Hz,benzylic); 2.89-3.03 (2H,m,H2'); 2.77 (1H,dd,J=9,13,benzylic); 1.20 (3H,t,J=7 Hz,H2').

Preparation D: Preparation of (4S)-N-[(2S,3R)-2-methyl-3-hydroxyhexanoyl]-4-benzyl-2-oxazolidinone

- 10 A dry, 2 L three-necked round bottomed flask equipped with a 500 mL addition funnel, a low-temperature thermometer, and a stir bar was charged with 19.84 g of N-propionyl-oxazolidinone, capped with septa and flushed with nitrogen. Anhydrous dichloromethane (100 mL) was added by cannula, and the resulting solution was cooled to -65°C in a bath of dry ice/isopropanol. The addition funnel
15 was charged by cannula with 100 mL of dibutylboron triflate (1.0 M in dichloromethane), which was added in a slow stream to the reaction. Triethylamine (15.6 mL) was added dropwise by syringe, keeping the reaction temperature below -10°C. The reaction was then transferred to an ice bath and allowed to stir at 0°C for 30 min. After that period, the reaction was placed back into the dry ice/isopropanol bath
20 and allowed to cool to -65°C. Butyraldehyde (8.6 mL) was added rapidly by syringe, and the reaction was allowed to stir for 30 min.

- The reaction was transferred to an ice bath and the addition funnel was charged with 100 mL of a 1 M aqueous phosphate solution, pH 7.0 (the phosphate solution is comprised of equal molar amounts of mono- and dibasic potassium
25 phosphate). The phosphate solution was added as quickly as possible while keeping the reaction temperature below 10°C. The addition funnel was then charged with 300 mL methanol which was added as quickly as possible while keeping the reaction temperature below 10°C. Finally, the addition funnel was charged with 300 mL of 2:1 methanol:30% hydrogen peroxide. This was added dropwise to ensure that the
30 temperature was kept below 10°C. The reaction was stirred for one hr. after completion of addition. The solvent was then removed on a rotary evaporator until a slurry remained. The slurry was extracted 4 times with 500 mL portions of ethyl ether. The combined organic extracts were washed with 250 mL each of saturated aqueous sodium bicarbonate and brine. The extract was then dried with MgSO₄, filtered, and
35 concentrated to give a slightly yellow oil. The material was then chromatographed on SiO₂ using 2:1 hexanes:ethyl acetate (product R_f = 0.4) resulting in 22.0 g (85% yield) of title compound as a colorless oil.

5 APCI-MS: m/z 306 (MH⁺); ¹H-NMR (360 MHz, CDCl₃): 7.2-7.4 (5H,m, phenyl); 4.71 (1H,m,H4); 4.17-4.25 (2H,m,H5); 3.96 (1H,m,H3'); 3.77 (1H,dq,J=2.5,7 Hz, H2'); 3.26 (1H,dd,J=4,13 Hz,benzylic); 2.79 (1H,dd,J=9,13 Hz,benzylic); 1.5-1.6 (2H,m,H4'); 1.3-1.5 (2H,m,H5'); 1.27 (3H,d,J=7 Hz,2'-Me); 0.94 (3H,t,J=7 Hz,H6').

10

Preparation E: Preparation of (2S,3R)-2-methyl-3-hydroxyhexanoate N-acetylcysteamine thioester

N-acetylcysteamine was distilled at 130°C/7 mm Hg to give a colorless liquid at room temperature. A dry, 1 L three-necked round bottomed flask equipped with a
15 500 mL addition funnel and a stir bar was capped with septa and flushed with nitrogen. The flask was then charged with 10.7 mL of N-acetylcysteamine by syringe and with 400 mL of anhydrous THF by cannula. The mixture was cooled with a MeOH/ice bath. Butyllithium (64 mL of 1.6 M in hexanes) was added dropwise by syringe, resulting in formation of a white precipitate. After stirring for 30min.,
20 trimethylaluminum (51 mL of 2.0 M in hexanes) was added dropwise by syringe. The reaction became clear after addition of trimethylaluminum and was allowed to stir an additional 30 min. During this period, 20.5 g (0.068 mol) of (4S)-N-[(2S,3R)-2-methyl-3-hydroxyhexanoyl]-4-benzyl-2-oxazolidinone was put under a blanket of nitrogen and dissolved in 100 mL of anhydrous THF; this solution was then
25 transferred in a slow stream by cannula into the reaction. The resulting reaction mixture turned a yellow-green color and was allowed to stir for 1 hr. The reaction was finished when the starting material could no longer be seen by thin-layer chromatographic analysis (ca. 1 hr.).

The reaction was treated with enough saturated oxalic acid to give a neutral
30 reaction with pH paper (approximately 90 mL). The solvents were then removed on a rotary evaporator to give a white slurry. The slurry was extracted six times with 250 mL portions of ethyl ether. The organic extracts were combined and washed with brine, dried with MgSO₄, filtered, and concentrated to give a slightly yellow oil. The thioester product was purified by flash chromatography on SiO₂ using 1:1
35 hexanes:EtOAc until the elution of 4-benzyl-2-oxazolidinone. At that point, the solvent system was switched to 100% EtOAc to give pure fractions of diketide thioester. The product fractions were combined and concentrated to give 14.9 g (89%

5 yield) of title compound. This compound is referred to as the propyl diketide thioester in Example 2.

APCI-MS: m/z 248 (MH⁺); ¹H-NMR (360 MHz, CDCl₃): 5.8 (br s, 1H); 3.94 (dt, 1H), 3.46 (m, 2H), 3.03 (dt, 2H), 2.71 (dq, 1H), 1.97 (s, 3H), 1.50 (m, 2H), 1.37 (m, 2H), 1.21 (d, 3H), 0.94 (t, 3H).

10

Preparation F: Preparation of (4S)-N-[(2S,3R)-2-methyl-3-hydroxy-4-pentenoyl]-4-benzyl-2-oxazolidinone

A dry, 2 L three-necked round bottomed flask equipped with a 500 mL addition funnel, a low-temperature thermometer, and a stir bar was charged with 20.0 g of propionyl oxazolidinone A, capped with septa and flushed with nitrogen. 15 Anhydrous dichloromethane (100 ml) was added and the resulting solution was cooled to -15°C in a bath of methanol/ice. Dibutylboron triflate (100 mL of 1.0 M in dichloromethane) was added in a slow stream via the addition funnel at such a rate as to keep the reaction temperature below 3°C. Diisopropylethylamine (17.9 mL) was 20 added dropwise by syringe, again keeping the internal temperature below 3°C. The reaction was then cooled to -65°C using a dry ice/isopropanol bath. Acrolein was added over 5 min. by syringe. The reaction was allowed to stir for 30 min. after completion of addition.

The reaction was then transferred to an ice bath and the addition funnel was 25 charged with 120 mL (0.1 mol) of a 1 M aqueous phosphate solution, pH 7.0 (the phosphate solution is comprised of equal molar amounts of mono- and dibasic phosphate). The phosphate solution was added as quickly as possible while keeping the reaction temperature below 10°C. The addition funnel was then charged with 400 mL of methanol that were added as quickly as possible while keeping the reaction 30 temperature below 10°C. Finally, the addition funnel was charged with 400 mL of 2:1 methanol:30% hydrogen peroxide by initial dropwise addition to keep the temperature below 10°C. The reaction was stirred for one hour. The solvent was removed using a rotary evaporator, leaving a slurry. The slurry was extracted 4 times with 500 mL portions of ethyl ether. The organic extracts were combined and washed with 250 mL 35 each of saturated sodium bicarbonate and brine, then dried with MgSO₄, filtered, and concentrated to give a slightly yellow oil. Trituration with hexane induced

5 crystallization. Recrystallization from ether by addition of hexane resulted in 13.67 g (55% yield) of product.

¹H-NMR (360 MHz, CDCl₃): 7.2-7.4 (m, 5H); 5.86 (ddd, 1H), 5.35 (dt, 1H), 5.22 (dt, 1H), 4.71 (m, 1H), 4.51 (m, 1H), 4.21 (m, 2H), 3.89 (dq, 1H), 3.26 (dd, 1H), 2.80 (dd, 1H), 1.25 (d, 3H).

10

Preparation G: Preparation of (2S,3R)-2-methyl-3-hydroxy-4-pentenoate
N-acetylcysteamine thioester

N-acetylcysteamine was distilled at 130°C/7 mm Hg to give a colorless liquid at room temperature. A dry, 1 L three-necked round bottomed flask equipped with a
15 500 mL addition funnel and a stir bar was capped with septa and flushed with nitrogen. The flask was then charged with 7.5 mL of N-acetylcysteamine by syringe and with 500 mL of anhydrous THF by cannula. The reaction was then cooled with a MeOH/ice bath. Butyllithium (44 mL of 1.6 M in hexane) was added dropwise by syringe. A white precipitate formed as the n-BuLi was added. After stirring for
20 30 min., 35.5 mL (0.071 mol) of trimethylaluminum (2.0 M in hexane) were added drop-wise by syringe. The reaction became clear after addition of trimethylaluminum and was allowed to stir an additional 30 min. (4S)-N-[(2S,3R)-2-methyl-3-hydroxy-4-pentenoyl]-4-benzyl-2-oxazolidinone from Preparation F (13.6 g) was put under a blanket of nitrogen, dissolved in 50 mL of anhydrous THF, and this solution was then
25 transferred in a slow stream by cannula into the reaction. The resulting reaction mixture turned a yellow-green color and was allowed to stir for 1 hr. The reaction was judged to be finished when starting material could no longer be seen by thin-layer chromatography (ca. 30 min.).

Enough saturated oxalic acid was added to give a neutral reaction with pH
30 paper (approximately 60 mL). The solvents were then removed by rotary evaporator to give a white slurry. The slurry was extracted six times with 250 mL portions of ethyl ether. The organic extracts were combined, washed with brine, dried with MgSO₄, filtered, and concentrated to give a slightly yellow oil. The thioester was then purified by flash chromatography on SiO₂. The column was run with 1:1
35 hexanes:ethyl acetate until the elution of oxazolidinone. At that point, the eluent was switched to 100% ethyl acetate to give pure fractions of product. The fractions were

5 combined and concentrated to give 7.7 g (71% yield) of title compound product. This product is referred to as the vinyl diketide thioester in Example 2.

¹H-NMR (360 MHz, CDCl₃): 5.82 (ddd, 1H), 5.78 (br s, 1H), 5.32 (dt, 1H), 5.21 (dt, 1H), 4.47 (m, 1H), 3.45 (m, 2H), 3.04 (m, 2H), 2.81 (dq, 1H), 1.96 (s, 3H), 1.22 (d, 3H).

10

Example 2

Preparation of Erythronolides

Preparation A: Preparation of 14,15-dehydro-6-deoxyerythronolide B

Streptomyces coelicolor CH999/pJRJ2 is described in U.S. patent application
15 Serial Nos. 08/896,323, filed 17 July 1997, and 08/675,817, filed 5 July 1996, each of which is incorporated herein by reference. Plasmid pJRJ2 encodes a mutated form of DEBS in which the ketosynthase domain of module 1 (KS1) has been inactivated via mutagenesis (KS1^o). S. coelicolor strains comprising this plasmid that are fed the vinyl diketide thioester prepared in accordance with Example 1 produce 14,15-
20 dehydro-6-deoxyerythronolide B:

Twenty isolates of S. coelicolor CH999/pJRJ2 were tested for their ability to convert the vinyl diketide thioester into 14,15-dehydro-6-deoxyerythronolide B. A frozen spore stock was diluted, plated on R2YE agar plates containing 50 mg/L thiostrepton, and grown at 30°C for 5 days to obtain single colonies. Each liter of
25 R2YE medium contains 103 g sucrose, 10 g glucose, 10.12 g MgCl₂·6H₂O, 0.25 g K₂SO₄, 0.1 g casamino acids, 5 g yeast extract, 5.73 g TES (N-tris[hydroxymethyl]methyl-2-aminoethane sulfonic acid, from Sigma) buffer, 22 g agar (when included), and 2 mL trace elements solution. After autoclaving, 10 mL 5 g/L (0.5%) KH₂PO₄, 8 mL 2.5 M CaCl₂·2H₂O, 15 mL 200 g/L (20%) L-proline, and 7
30 mL 1 N NaOH were added. Each liter of trace elements solution contains 1 mg ZnSO₄, 1 mg FeSO₄, 1 mg MnCl₂, and 1 mg CaCl₂. TES was omitted from R2YE media when cultures were grown in pH-controlled bioreactors:

The colonies were patched onto secondary plates for amplification, then spread on fresh R2YE agar plates containing 50 mg/L thiostrepton to create mycelial lawns.
35 Diketide feeding of Streptomyces coelicolor CH999/pJRJ2 to these lawns was performed as previously described (see the patent applications cited supra). The 14,15-dehydro-6-deoxyerythronolide B produced in these cultures was isolated by

5 homogenization and ethyl acetate extraction of the agar on which the culture was grown. Reversed phase HPLC/MS analysis of these extracts was performed using a Beckman 127s solvent module equipped with a Beckman Ultrasphere ODS column (4.6 mm x 150 mm) and a gradient of water to acetonitrile as the mobile phase. The 14,15-dehydro-6-deoxyerythronolide B was identified by mass spectrometry (PESciex
10 API100LC) and quantitated using an evaporative light scattering detector (Alltech 500ELSD). High-producing isolates propagated on plates and as frozen spore suspensions were retested to determine their stability during storage.

Of the twenty isolates of *Streptomyces coelicolor* CH999/pJRJ2 tested for ability to convert the vinyl diketide thioester into 14,15-dehydro-6-
15 deoxyerythronolide B, five isolates were non-producers, and one isolate produced >6 mg/L of product. The high-producing isolate was propagated both on agar medium (restreaking every ~10 days) and as a frozen spore stock. Reisolation of the strain resulted in considerable isolate-to-isolate variation when the strain was stored as a frozen spore stock. When the strain was stored at room temperature on R2YE agar this
20 variability was not observed. Segregation of the production ability of a strain during storage as a frozen spore suspension has been previously observed, although the mechanism by which it occurs is unknown.

Propagation of the strain on agar media and as a frozen mycelial suspension retain an isolate's production capability. Consequently, a cell bank was prepared by
25 inoculating approximately 10 mm² mycelial patches into 50 mL of R2YE containing 50 µg/mL thiostrepton and shaken (series 25 New Brunswick coffin shaker) at 200-250 rpm/28-30°C in a 250 mL baffled flask for 48 hr. The cells were microscopically examined, 25 mL of 90% glycerol was mixed into the culture, and 1 mL aliquots were frozen in liquid nitrogen and stored at -80° C. This procedure was used for storage of
30 both *Streptomyces coelicolor* and *Saccharopolyspora erythraea* strains.

14,15-dehydro-6-deoxyerythronolide B can be produced in shake flasks. A seed culture of *Streptomyces coelicolor* CH999/pJRJ2 was made by adding 1 mL of frozen stock to 50 mL of R2YE containing 50 µg/mL thiostrepton and ~1 mL/L antifoam B (Baker). Seed cultures of *S. coelicolor* K39-02/pJRJ2 optionally may
35 contain 50 µg/mL apramycin. The culture was shaken at 200-250 rpm at 28-30°C for ~48 hr (Series 25 New Brunswick coffin shaker). A production culture was made by inoculating 10 mL of the seed culture into 500 mL of SO1 medium (optionally, one

5 may use R6 medium with no buffer) containing 50 µg/mL thiostrepton. Each liter of SO1 medium contained 51.5 g sucrose, 0.25 g K₂SO₄, 0.1 g casamino acids, 5 g yeast extract, 5.73 g TES buffer, 0.96 g sodium propionate, and 2 mL trace elements-
solution. After autoclaving, 10 mL 0.5 % (5 g/L) KH₂PO₄, 8 mL 2.5 M CaCl₂•6H₂O,
7.5 mL 20% (200 g/L) L-proline, and 7 mL 1 N NaOH were added. TES was omitted
10 from SO1 media when cultures were grown in pH-controlled bioreactors.

R6 medium was also used for production cultures. Each liter of R6 medium contained 103 g sucrose, 0.25 g K₂SO₄, 10.12 g MgCl₂•6H₂O, 0.1 g casamino acids, 5 g yeast extract, 5.73g TES buffer, 0.96 g sodium propionate, and 2 mL trace elements solution. After autoclaving, 10 mL 0.5% KH₂PO₄, 8 mL 2.5 M CaCl₂•2H₂O, 15 mL
15 20% L-proline, and 7 mL 1 N NaOH were added. TES was omitted from R6 media when cultures were grown in pH-controlled bioreactors.

The culture was grown for 36-48 hr. at 200-250 rpm/28-30°C. The culture was then supplemented with 4-pentynoic acid (Fluka, 25 mg/L) and 1 mM vinyl diketide thioester (3 mL of 4.67 mg/mL diketide in 10% DMSO (Sigma)), and grown for 4
20 additional days. For diketide feeding in R6 medium, diketide was typically added 24-48 hrs. after inoculation, when the glucose level dropped to 0.5 g/L or lower; glucose concentration can be analyzed to time the feeding more exactly. 14,15-dehydro-6-deoxyerythronolide B was recovered from the culture by solid phase extraction with XAD resin and elution with ethanol.

25 For large-scale preparation of 14,15-dehydro-6-deoxyerythronolide B, a seed culture of *Streptomyces coelicolor* K39-02/pJRJ2 was made by inoculating 1 mL of frozen mycelium into a 2.8 L baffled flask containing 500 mL of R2YE, optionally 50 µg/mL apramycin, 50 µg/mL thiostrepton, and 1mL/L antifoam B, and shaking at 150-200 rpm/28-30°C for about 2 days (Innova floor shaker). A 10 L stirred tank
30 bioreactor (B. Braun A-10) was prepared, filled with 10 L of R6 medium, autoclaved at 121°C for 30 min., allowed to cool, and then inoculated with 500 mL (2%) of seed culture.

Temperature was maintained at 30°C with agitation provided by 3 rushton impellers at 500-750 rpm, aeration at 0.5 – 2 L/min., and pH controlled at 7.00 via
35 automatic addition of 1 N NaOH or 1 N H₂SO₄. Glucose consumption, dissolved oxygen, pH, and cell mass were monitored. When the glucose concentration dropped below 0.1 g/L, the culture was supplemented with 4-pentynoic acid (25-50 µg/mL)

5 and 2.5 g of the vinyl diketide thioester in 50 mL of DMSO. Controlled feeding of glucose maintained a glucose concentration of 0 – 2 g/L (target of 0.5 g/L). Titrers of 14,15-dehydro-6-deoxyerythronolide B were monitored by HPLC/MS, and the culture was harvested by centrifugation when a maximum titer was reached. The procedure was scaled to 100 L using a BioLafitte 150 L bioreactor.

10 The 14,15-dehydro-6-deoxyerythronolide B was purified by solid phase extraction. The fermentation broth was chilled to 4 – 15°C, and ethanol was added (0.1 L/L broth). The broth was clarified by centrifugation and loaded onto an XAD-16 resin (Rohm and Haas) column (1 kg XAD/1 g 14,15-dehydro-6-deoxyerythronolide B) at a flow rate of 2-4 mL/cm²-min. The loaded resin was washed with 2 column
15 volumes of 15% (v/v) ethanol in water and the 14,15-dehydro-6-deoxyerythronolide B was eluted from the resin with acetone and collected in ½ column volume fractions. The fractions containing 14,15-dehydro-6-deoxyerythronolide B were identified by thin-layer chromatography (ethyl acetate:hexanes 1:1) and HPLC/MS.

The acetone fractions containing 14,15-dehydro-6-deoxyerythronolide B were
20 pooled, and the volatiles were removed under reduced pressure. The resulting aqueous mixture is extracted with ethyl acetate. The ethyl acetate extract was washed with saturated NaHCO₃ and brine solutions, dried over sodium or magnesium sulfate, filtered, and concentrated to dryness under reduced pressure. The crude material was purified by chromatography on silica gel using a gradient of hexanes and ethyl
25 acetate. Fractions containing the product were pooled and concentrated to a yellow oil that spontaneously crystallized. Recrystallization from ether-hexane gave pure 14,15-dehydro-6-deoxyerythronolide B. Mass spectrometry shows [M+H] = 385. ¹³C-NMR (CDCl₃, 100 MHz): 213.67 (C9), 177.51 (C1), 134.80 (C14), 116.58 (C15), 79.40 (C3), 76.47 (C5), 74.11 (C13), 70.84 (C11), 43.80 (C2), 43.16 (C10), 41.48 (C12),
30 39.58 (C8), 37.61 (C7), 37.42 (C4), 35.56 (C6), 16.60 (6Me), 14.55 (2Me), 13.34 (8Me), 9.20 (12Me), 6.91 (4Me), 6.30 (10Me).

Preparation B: Preparation of 15-methyl-6-deoxyerythronolide B

A high-producing isolate of *Streptomyces coelicolor* K39-02/pJRJ2 was used
35 to produce 15-methyl-6-deoxyerythronolide B in shake flasks. A seed culture of *Streptomyces coelicolor* K39-02/pJRJ2 was made by adding 1 mL of frozen stock to 50 mL of R2YE containing 50 µg/mL thiostrepton (and, optionally, apramycin). The

5 culture was shaken at 200-250 rpm at 28-30°C for 36-48 hr. A production culture was made by inoculating 1 mL of the seed culture into 50 mL of SO1 or R6 medium containing 50 µg/mL thiostrepton (and, optionally, apramycin). Antifoam was added at 1 mL/L. The culture was grown for 36-48 hr. at 200-250 rpm/28-30°C. The culture was supplemented with 4-pentynoic acid (Fluka, 25-50 mg/L) and 1 mM propyl
10 diketide thioester (3 mL of 4.67 mg/mL diketide in 10% DMSO (Sigma)), and grown for 4-7 additional days. 15-methyl-6-deoxyerythronolide B was recovered from the culture by extraction with ethyl acetate when maximum titer was reached.

For large-scale preparation of 15-methyl-6-deoxyerythronolide B, a seed culture of *Streptomyces coelicolor* K39-02/pJRJ2 was made by inoculating 1 mL of
15 frozen mycelium into a 2.8 L baffled flask containing 500 mL of R2YE and shaking at 150-200 rpm/28-30°C for 2 days. A 10 L stirred tank bioreactor was prepared, filled with 10 L of SO1 or R6 medium, autoclaved at 121°C for 30 min., allowed to cool, and then inoculated with 400-500 mL of seed culture.

Temperature was maintained at 28-30°C with agitation provided by 3 rushton
20 impellers at 500-750 rpm, aeration at ~1 L/min., and pH controlled at 7.00 via automatic addition of 1 N NaOH or 1 N H₂SO₄. Glucose consumption, dissolved oxygen, pH, and cell mass were monitored. When the glucose concentration dropped below 0.1 g/L, the culture was supplemented with 4-pentynoic acid (25 µg/mL) and 2.5 g of the propyl diketide thioester in 50 mL of DMSO. Controlled feeding of
25 glucose maintained a glucose concentration of ~0.5 g/L. Titrers of 15-methyl-6-deoxyerythronolide B were monitored by HPLC/MS, and the culture was harvested by centrifugation when a maximum titer was reached. The procedure was scaled to 100 L using a BioLafitte 150 L bioreactor.

The 15-methyl-6-deoxyerythronolide B was purified by solid phase extraction.
30 Fermentation broth was cooled to 4 – 15°C, and ethanol was added (0.1 L/L broth). The broth was clarified by centrifugation and loaded onto an XAD-16 resin (Rohm and Haas) column (1 kg XAD/1 g 15-methyl-6-deoxyerythronolide B) at a flow rate of 2-4 mL/cm²-min. The loaded resin was washed with 2 column volumes of 15% (v/v) ethanol in water and the 15-methyl-6-deoxyerythronolide B was eluted from the
35 resin with acetone and collected in ½ column volume fractions. The fractions containing 15-methyl-6-deoxyerythronolide B were identified by thin-layer chromatography (ethyl acetate:hexanes 1:1) and HPLC/MS.

5 The acetone fractions containing 15-methyl-6-deoxyerythronolide B were pooled, and the volatiles were removed under reduced pressure. The resulting aqueous mixture is extracted with ethyl acetate. The ethyl acetate extract was washed with saturated NaH_2CO_3 and brine solutions, dried over sodium or magnesium sulfate, filtered, and concentrated to dryness under reduced pressure. The crude material was
10 purified by chromatography on silica gel using a gradient of hexanes and ethyl acetate. Fractions containing the product were pooled and concentrated to a yellow oil that spontaneously crystallized. Recrystallization from ether-hexane gave pure 15-methyl-6-deoxyerythronolide B. Mass spectrometry shows $[\text{M}+\text{H}] = 401$.

15 Preparation C: Preparation of 14-nor-6-deoxyerythronolide B

U.S. Patent No. 5,712,146, incorporated herein by reference, describes the preparation of a recombinant host cell, *Streptomyces coelicolor* CH999/pCK7. The patent reports that, when the recombinant strain is grown on R2YE medium, the strain produces a mixture of 6-deoxyerythronolide B and 14-nor-6-deoxyerythronolide B
20 (also known as 8,8a-deoxyoleandolide). A related strain, *S. coelicolor* 27-26/pKOS011-26 contains a modified pCK7 plasmid and a recombinant ptpA gene.

A high-producing isolate of *Streptomyces coelicolor* 27-26/pKOS011-26 was used to produce 14-nor-6-deoxyerythronolide B in shake flasks. A seed culture of *Streptomyces coelicolor* 27-26/pKOS011-26 was made by adding 1 mL of frozen
25 stock to 50 mL of R2YE containing 50 $\mu\text{g}/\text{mL}$ of thiostrepton (and, optionally, apramycin). The culture was shaken at 200-250 rpm at 28-30°C for 36-48 hr. A production culture was made by inoculating 1 mL of the seed culture into 50 mL of SO1 medium containing 50 $\mu\text{g}/\text{mL}$ thiostrepton (and, optionally, apramycin). The culture was grown for 36-48 hr. at 200-250 rpm/28-30°C. The culture was
30 supplemented with 4-pentynoic acid (Fluka, 25-50 mg/L) and grown for 4 additional days. 14-nor-6-deoxyerythronolide B was recovered from the culture by extraction with ethyl acetate.

For large-scale preparation of 14-nor-6-deoxyerythronolide B, a seed culture of *Streptomyces coelicolor* 27-26/pKOS011-26 was made by inoculating 1 mL of
35 frozen mycelium into a 2.8 L baffled flask containing 500 mL of R2YE and shaking at 150-200 rpm/28-30°C for 36-48 hr. A 10 L stirred tank bioreactor was prepared, filled with 10 L of R2YE medium without glucose, autoclaved at 121°C for 30 min.,

5 allowed to cool, and then inoculated with 400-500 mL of seed culture. Antifoam was added at 1 mL/L.

Temperature was maintained at 28-30°C with agitation provided by 3 rushton impellers at 500-750 rpm, aeration at ~1 L/min., and pH controlled at 7.00 via automatic addition of 1 N NaOH or 1 N H₂SO₄. Glucose consumption, dissolved
10 oxygen, pH, and cell mass were monitored. Controlled feeding of glucose maintained a glucose concentration of ~0.5 g/L. Titrers of 14-nor-6-deoxyerythronolide B were monitored by HPLC/MS, and the culture was harvested by centrifugation when a maximum titer was reached. The procedure was scaled to 100 L using a BioLafitte 150 L bioreactor.

15 The 14-nor-6-deoxyerythronolide B was purified by solid phase extraction. Fermentation broth was chilled to 4 - 15°C, and ethanol was added (0.1 L/L broth). The broth was clarified by centrifugation and loaded onto an XAD-16 resin (Rohm and Haas) column (1 kg XAD/1 g 14-nor-6-deoxyerythronolide B) at a flow rate of 2-4 mL/cm²-min. The loaded resin was washed with 2 column volumes of 15% (v/v)
20 ethanol in water and the 14-nor-6-deoxyerythronolide B was eluted from the resin with acetone and collected in ½ column volume fractions. The fractions containing 14-nor-6-deoxyerythronolide B were identified by thin-layer chromatography (ethyl acetate:hexanes 1:1) and HPLC/MS.

The acetone fractions containing 14-nor-6-deoxyerythronolide B were pooled,
25 and the volatiles were removed under reduced pressure. The resulting aqueous mixture is extracted with ethyl acetate. The ethyl acetate extract was washed with saturated NaH₂CO₃ and brine solutions, dried over sodium or magnesium sulfate, filtered, and concentrated to dryness under reduced pressure. The crude material was purified by flash chromatography using SiO₂ columns developed with ethyl
30 acetate/hexanes. Recrystallization from ether-hexane gave pure 15-methyl-6-deoxyerythronolide B. Mass spectrometry shows [M+H] = 373.

Example 3

Preparation of Erythromycins

The 6-dEB derivative compounds produced in Example 2, Preparations A – C are converted to erythromycin derivatives using a recombinant strain of *Saccharopolyspora erythraea*. For production of erythromycins having both the 6 and 12 hydroxyl groups, the *S. erythraea* strain used was K40-67. This strain was created by transforming an *S. erythraea* strain capable of producing high levels of erythromycin A with a pWHM3-derived plasmid comprising a mutated *eryA1* sequence encoding an inactivated KS1 domain. By homologous recombination, the resulting transformants were rendered incapable of producing 6-deoxyerythronolide B. For production of erythromycin derivatives having only the 12-hydroxyl group, the *S. erythraea* strain used was K39-07. This strain was constructed from strain K40-67 by disruption of the *eryF* hydroxylase gene. Both strains were fermented under substantially similar conditions, as described below.

Fermentations were conducted in 10 L (and 150 L) bioreactors. A 1 mL aliquot of frozen *S. erythraea* K40-67 mycelium was used to inoculate a seed culture in 500 mL of R2YE medium. The culture was shaken at 150-200 rpm/28-30°C in a 2.8 L baffled Fernbach flask for ~48 hr. A 10 L stirred tank bioreactor was prepared, filled with 10 L of R2YE medium (70 L for the 150 L fermentation), autoclaved at 121°C for 45 min., allowed to cool, and then inoculated with 200 mL (1.4 L for the 150 L fermentation) of seed culture. Temperature was maintained at 28-30°C with agitation provided by 2 rushton impellers at 500-700 rpm, aeration at ~1 L/min., and pH controlled at 7.20 via automatic addition of 1 N NaOH or 1 N H₂SO₄. Foam was suppressed by addition of antifoam at 1 mL/L. The pH was controlled to avoid potential product degradation into enol ether and spiroketal. Sucrose consumption, glucose evolution, dissolved oxygen, pH, and absorbance at 600 nm (cell mass) were monitored. After 24-36 hr., the culture was fed 300 mg (1.62 g for the 150 L fermentation) of a 6-dEB derivative compound prepared in accordance with Preparations A – C of this Example dissolved in 3 mL (15 mL for the 150 L fermentation) of 100% ethanol. Fermentation continued for ~68-85 additional hr., and the fermentation broth was harvested by centrifugation. Titrers of erythromycin A, B, C, and D analogs during the course of the fermentation were determined by electrospray MS analysis.

5 The compounds produced were purified by solid phase extraction. Fermentation broth was brought to pH 8.0 by addition of NaOH and chilled to 4 – 15°C, and ethanol was added (0.1 L/L broth). The broth was clarified by centrifugation and loaded onto an XAD-16 resin (Rohm and Haas) column (1 kg XAD/1 g erythromycin derivative) at a flow rate of 2-4 mL/cm²-min. The loaded resin
10 was washed with 2 column volumes of 15% (v/v) ethanol in water and the erythromycin derivative was eluted from the resin with acetone and collected in ½ column volume fractions. The fractions containing the erythromycin derivative were identified by thin-layer chromatography and HPLC/MS.

 The acetone fractions containing the erythromycin derivative were pooled, and
15 the volatiles were removed under reduced pressure. The resulting aqueous mixture was extracted with ethyl acetate. The ethyl acetate extract was washed with saturated NaH₂CO₃ and brine solutions, dried over sodium or magnesium sulfate, filtered, and concentrated to dryness under reduced pressure. The crude material was purified by flash chromatography (methylene chloride/methanol/triethylamine). This material
20 served as starting material for the chemical derivatization procedures described in the following examples. Pure products may be obtained through the use of centrifugal countercurrent distribution (e.g., using an Ito Coil Planet Centrifuge as described in WO 91/16334, incorporated herein by reference).

 The compounds produced by this methodology were: (i) 14-nor erythromycin
25 A; (ii) 14,15-dehydro-erythromycin A; (iii) 15-methyl-erythromycin A; (iv) 14-nor-6-deoxy-erythromycin A; (v) 14,15-dehydro-6-deoxy-erythromycin A; and (vi) 15-methyl-6-deoxy-erythromycin A. When used to make 3-descladinose-3-oxo-derivatives, the erythromycin A derivatives were not separated from the erythromycin C derivatives; instead, mixtures of the A and C compounds were used as starting
30 materials for chemical derivatization.

Example 4

Preparation A: 14-norerythromycin A 9-oxime

 A solution of 14-norerythromycin A (0.621 g, 86% pure), hydroxylamine (0.5
35 ml of 50% aqueous solution) and acetic acid (0.2 ml) in isopropanol (2 ml) was kept at 50°C for 22 hours. It was extracted with chloroform/ethanol (3/2), washed with sodium bicarbonate, brine, and dried over MgSO₄. Filtration and evaporation *in*

5 *vacuo* yielded a crude product (0.65 g) as a white solid which was used directly for next transformation.

Preparation B: 14-norerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime

To a solution of above crude 14-noreythromycin A 9-oxime (0.65 g) and 1,1-
10 diisopropoxy-cyclohexanone (0.95 ml) in methylene chloride (2 ml) was added pyridinium *p*-toluenesulfonate (PPTS) (0.333 g) in methylene chloride (2 ml). After stirring overnight, the mixture was extracted (chloroform/ethanol 3:2), washed (NaHCO₃-H₂O, brine), and dried (MgSO₄). After filtration and evaporation *in vacuo*, the crude product was repeatedly driven with toluene and isopropanol to yield 0.74 g
15 of product, which was used directly for next reaction.

Preparation C: 2',4''-bis(O-trimethylsilyl)-14-norerythromycin A 9-[O-(1-isopropoxy-cyclohexyl)]oxime

To a solution of 14-norerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime
20 (0.74 g) in methylene chloride (6 ml) was added a solution of trimethylsilyl imidazole (0.33 ml) and trimethylsilyl chloride (0.18 ml) in methylene chloride (2 ml) at 0°C. After 5 minute stirring, ethyl acetate was added, washed (NaHCO₃-H₂O, brine), and dried (MgSO₄). Flash chromatography on silica gel (10:1 hexanes:acetone, 1% triethylamine) afforded pure product as a white solid (0.50 g). Mass spectrometry
25 reveals [M+H⁺] = 1020.

Preparation D: 2',4''-bis-(O-trimethylsilyl)-6-O-methyl-14-norerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime

A solution of 2',4''-bis-O-trimethylsilyl-14-norerythromycin A 9-[O-(1-isopropoxy-cyclohexyl)]oxime (0.3 g, 0.29 mmol) in 1:1
30 methylsulfoxide/tetrahydrofuran (DMSO/THF) (1.4 ml) was treated with 0.3 ml of a 2 M solution of methyl bromide in ether and cooled to 10°C. A mixture of 1 M solution of potassium *tert*-butoxide in THF (0.6 ml) and DMSO (0.6 ml) was added over 6 hours using a syringe pump. The reaction was then diluted with ethyl acetate, washed
35 with saturated NaHCO₃, brine, and dried over MgSO₄. Filtration and evaporation *in vacuo* yielded the product (0.29 g) as a white solid. Mass spectrometry reveals [M+H⁺] = 1034.

5

Preparation E: 6-O-methyl-14-norerythromycin A 9-oxime

A mixture of 6-*O*-methyl-2'',4''-*bis-O*-trimethylsilyl-14-norerythromycin A 9-[*O*-(1-isopropoxycyclohexyl)] oxime (0.29 g), acetic acid (3.6 ml), acetonitrile (6 ml) and water (3 ml) was stirred at ambient temperature for 4.5 hours. The mixture was
10 driven to dryness using toluene to give a crude product as white solid (0.24 g), which was used directly for next step without further purification.

Preparation F: 6-O-methyl-14-norerythromycin A

A mixture of 6-*O*-methyl-14-norerythromycin A 9-oxime (0.24 g), sodium
15 hydrosulfite (0.45 g, 85% pure), water (3 ml), ethanol (3 ml) and formic acid (0.07 ml) was kept at 85°C for 8 hours. The reaction was brought to pH 8 with 1 N NaOH and extracted with ethyl acetate. The organic extract was washed with brine, dried over MgSO₄, filtered, and concentrated to yield a crude product as a white solid (0.2 g). Mass spectrometry reveals [M+H⁺] = 735.

20

Example 5

Preparation A: 14,15-dehydroerythromycin A 9-oxime

A suspension of 14,15-dehydroerythromycin A (1.984 g, 47% purity, 1.2
25 mmol) in 6 mL of 2-propanol was treated with 1.97 mL of 50% aqueous hydroxylamine and stirred until dissolved. Acetic acid (0.62 mL) was added and the mixture was stirred for 25 hours at 50 °C. Upon cooling to ambient temperature, saturated NaHCO₃ was added and the mixture was concentrated en vacuo to remove isopropanol. The resulting aqueous mixture was extracted three times with 250-mL
30 portions of CHCl₃. The organic extracts were combined, washed with saturated NaHCO₃, water, and brine, then dried over MgSO₄, filtered, and concentrated to yield 0.92 g of product.

Preparation B: 14,15-dehydroerythromycin A 9-[*O*-(1-isopropoxycyclohexyl)]oxime

35 The oxime from (A) (0.92 g) was dissolved in 6.2 mL of CH₂Cl₂ and treated with 1,1-diisopropoxycyclohexane (1.23 g) and pyridinium p-toluenesulfonate (0.464 gm) for 15 hours at ambient temperature. The mixture was diluted with 160 mL of

- 5 CH₂Cl₂, then washed sequentially with saturated NaHCO₃, water, and brine. The organic phase was dried with MgSO₄, filtered, and evaporated to yield a brown syrup. Chromatography on silica gel (gradient from toluene to 1:1 toluene/acetone + 1% Et₃N) yielded 0.998 g of product.

10 Preparation C: 2',4''-bis(O-trimethylsilyl)-14,15-dehydroerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime

- A solution of 14,15-dehydroerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime (998 mg, 9.96) in 11.25 mL of CH₂Cl₂ was cooled on ice under inert atmosphere and treated with a solution of chlorotrimethylsilane (0.24 mL) and 1-trimethylsilylimidazole (0.44 mL). After 30 minutes, the reaction was diluted with 250 mL of ethyl acetate and washed sequentially with saturated NaHCO₃, water, and brine. The organic phase was dried with MgSO₄, filtered, and evaporated to yield 1.002 g of product.

20 Preparation D: 2',4''-bis(O-trimethylsilyl)-6-O-methyl-14,15-dehydroerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime

- A solution of 2',4''-bis-O-trimethylsilyl-14,15-dehydroerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime (1.00 g, 20.7 mmol) in 9.69 mL of 1:1 tetrahydrofuran/methylsulfoxide was cooled to 10 °C and treated with 0.97 mL of 2.0 M methyl bromide in ether under inert atmosphere. A mixture of methylsulfoxide (1.94 mL) and 1.0 M potassium *tert*-butoxide in tetrahydrofuran (1.94 mL) was added slowly. The reaction was monitored by thin-layer chromatography (silica gel, 10:1 toluene/acetone), and was judged complete after addition of 1.6 molar equivalents of base. The reaction was diluted with 200 mL of ethyl acetate and 70 mL of saturated NaHCO₃. The mixture was transferred to a separatory funnel, diluted with 850 mL of ethyl acetate and 280 mL of saturated NaHCO₃, then washed sequentially with water and brine. The organic phase was dried with MgSO₄, filtered through Celite, and evaporated to yield 21.2 g of crude 6-O-methyl-2',4''-bis-O-trimethylsilyl-14,15-dehydroerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime. This was carried on without further purification.

35 Preparation E: 6-O-methyl-14,15-dehydroerythromycin A 9-oxime

5 A solution of 6-O-methyl-2',4''-bis-O-trimethylsilyl-14,15-dehydroerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime (1.0 g) in 9.8 mL of 2:1 acetonitrile/water was treated with 5.3 mL of acetic acid, and stirred for 8 hours at ambient temperature. The mixture was concentrated en vacuo, then repeatedly concentrated after addition of toluene to yield 0.797 g of crude 6-O-methyl-14,15-dehydroerythromycin A 9-oxime.

Preparation F: 6-O-methyl-14,15-dehydroerythromycin A

A solution of 6-O-methyl-14,15-dehydroerythromycin A 9-oxime (0.797 g) and sodium hydrosulfite (85%, 1.02 g) in 7.5 mL of 1:1 ethanol/water was placed under inert atmosphere. Formic acid (0.186 mL) was added dropwise, and the mixture was stirred at 80 °C for 3 hours. After cooling to ambient temperature, the reaction was adjusted to pH 10 with 6 N NaOH and extracted three times with 150-mL portions of ethyl acetate. The organic extracts were combined and washed sequentially with saturated NaHCO₃, water, and brine. The organic phase was dried with MgSO₄, filtered, and evaporated to yield 0.68 g of 6-O-methyl-14,15-dehydroerythromycin A suitable for further conversion.

Example 6

25 Preparation A: 15-Methylerythromycin A 9-oxime

A suspension of 15-methylerythromycin A (20.0 g, 85% purity, 22.6 mmol) in 40 mL of 2-propanol was treated with 20.5 mL of 50% aqueous hydroxylamine and stirred until dissolved. Acetic acid (6.41 mL) was added and the mixture was stirred for 15 hours at 50 °C. Upon cooling to ambient temperature, saturated NaHCO₃ was added and the mixture was concentrated en vacuo to remove isopropanol. The resulting aqueous mixture was extracted three times with 250-mL portions of CHCl₃. The organic extracts were combined, washed with saturated NaHCO₃, water, and brine, then dried over MgSO₄, filtered, and concentrated to yield 20.5 g of crude product. Analysis by LC/MS revealed a 94:6 mixture of E and Z oximes, [M+H]⁺ = 764.

Preparation B: 15-Methylerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime

5 The crude oxime from above (20.5 g) was dissolved in 55 mL of CH₂Cl₂ and treated with 1,1-diisopropoxycyclohexane (27.3 mL) and pyridinium p-toluenesulfonate (9.8 gm) for 15 hours at ambient temperature. The mixture was diluted with 160 mL of CH₂Cl₂, then washed sequentially with saturated NaHCO₃, water, and brine. The organic phase was dried with MgSO₄, filtered, and evaporated to yield a brown syrup. Chromatography on silica gel (gradient from 2:1 to 3:2 hexanes/acetone + 1% Et₃N) yielded 18.0 g of product.

Preparation C: 2',4''-bis-O-trimethylsilyl-15-methylerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime

15 A solution of 15-Methylerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime (9.00 g, 9.96 mmol) in 25 mL of CH₂Cl₂ was cooled on ice under inert atmosphere and treated with a solution of chlorotrimethylsilane (1.89 mL) and 1-trimethylsilylimidazole (3.65 mL) in 8 mL of CH₂Cl₂. After 30 minutes, the reaction was diluted with 250 mL of ethyl acetate and washed sequentially with saturated NaHCO₃, water, and brine. The organic phase was dried with MgSO₄, filtered, and evaporated. The crude product was purified by silica gel chromatography (gradient from hexanes to 10:1 hexanes/acetone + 1% Et₃N), yielding 7.8 g of product.

Preparation D: 2',4''-bis-O-trimethylsilyl-6-O-methyl-15-methylerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime

25 A solution of 2',4''-bis-O-trimethylsilyl-15-methylerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime (21.7 g, 20.7 mmol) in 41.4 mL of tetrahydrofuran was cooled to 10 °C and treated with 41.4 mL of methylsulfoxide and 20.7 mL of 2.0 M methyl bromide in ether under inert atmosphere. A mixture of methylsulfoxide (41.4 mL) and 1.0 M potassium *tert*-butoxide in tetrahydrofuran (41.4 mL) was added at a rate of ca. 20 mL per hour. The reaction was monitored by thin-layer chromatography (silica gel, 10:1 toluene/acetone), and was judged complete after addition of 1.6 molar equivalents of base. The reaction was diluted with 200 mL of ethyl acetate and 70 mL of saturated NaHCO₃. The mixture was transferred to a separatory funnel, diluted with 850 mL of ethyl acetate and 280 mL of saturated NaHCO₃, then washed sequentially with water and brine. The organic phase was dried with MgSO₄, filtered through Celite, and evaporated to yield 21.2 g of crude 6-O-methyl-2',4''-bis-O-

- 5 trimethylsilyl-15-methylerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime. This was carried on without further purification.

Preparation E: 6-O-methyl-15-methylerythromycin A 9-oxime

- A solution of 6-O-methyl-2',4''-bis-O-trimethylsilyl-15-methylerythromycin
10 A 9-[O-(1-isopropoxycyclohexyl)]oxime (21.2 g) in 110 mL of acetonitrile was treated with 55 mL of water and 67 mL of acetic acid, and stirred for 8 hours at ambient temperature. The mixture was concentrated *en vacuo*, then repeatedly concentrated after addition of toluene to yield 19.7 g of 6-O-methyl-15-methylerythromycin A 9-oxime.

15

Preparation F: 6-O-methyl-15-methylerythromycin A

- A solution of 6-O-methyl-15-methylerythromycin A 9-oxime (19.7 g) and sodium hydrosulfite (85%, 23.1 g) in 280 mL of 1:1 ethanol/water was placed under inert atmosphere. Formic acid (3.75 mL) was added dropwise, and the mixture was
20 stirred at 80 °C for 4.5 hours. After cooling to ambient temperature, the reaction was treated with saturated NaHCO₃ and extracted three times with 400-mL portions of ethyl acetate. The organic extracts were combined and washed sequentially with saturated NaHCO₃, water, and brine. The organic phase was dried with MgSO₄, filtered, and evaporated to yield 15.1 g of 6-O-methyl-15-methylerythromycin A
25 suitable for further conversion.

Example 7

30

Preparation A: 6-O-methyl-3-descladinosyl-14-norerythromycin A

- A mixture of 6-O-methyl-14-norerythromycin A (77 mg), 0.073 ml of 12 N HCl and water (2 ml) was stirred at ambient temperature for 3 hours. The mixture was brought to pH 8 with 8 N KOH, and extracted with ethyl acetate. The organic
35 extract was washed with brine, dried with MgSO₄, filtered, and evaporated. The residue was chromatographed on silica gel (3:1/hexanes:acetone, 1% triethylamine) to give pure product as a white solid (42 mg). Mass spectrometry reveals [M+H⁺] = 576.

5

Preparation B: 2'-O-Acetyl-6-O-methyl-3-descladinosyl-14-norerythromycin A

A mixture of 6-O-methyl-3-descladinosyl-14-norerythromycin A (73 mg), potassium carbonate (20 mg), acetic anhydride (14 μ l) and acetone (1 ml) was stirred at ambient temperature for 18 hours. Ethyl acetate was added, washed with water and
10 brine, dried over MgSO_4 , filtered, and evaporated. The residue was chromatographed on silica gel (3:1/hexanes:acetone, 1% triethylamine) to yield the pure product (71 mg) as a white solid. Mass spectrometry reveals $[\text{M}+\text{H}^+] = 618$.

15

Preparation C: 2'-O-Acetyl-6-O-methyl-3-descladinosyl-3-oxo-14-norerythromycin

A

A solution of 2'-O-acetyl-6-O-methyl-3-descladinosyl-14-norerythromycin A (99 mg) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide(EDC) hydrochloride (206 mg) in dichloromethane (2 ml) was treated with DMSO (0.21 ml) and cooled to 5°C. A solution of pyridinium trifluoroacetate (208 mg) in dichloromethane (2 ml)
20 was added *via* a syringe pump in 4 hours. Ethyl acetate was then added, washed with saturated NaHCO_3 , water, brine, and dried over MgSO_4 , filtered, and evaporated. The residue was chromatographed on silica gel (3:1/hexanes:acetone, 1% triethylamine) to yield the pure product (94 mg) as a white solid. Mass spectrometry reveals $[\text{M}+\text{H}^+] = 616$.

25

Preparation D: 2'-O-Acetyl-6-O-methyl-3-descladinosyl-3-oxo-11-O-methanesulfonyl-14-norerythromycin A

To a solution of 2'-O-Acetyl-6-O-methyl-3-descladinosyl-3-oxo-14-norerythromycin A (93 mg) in dry pyridine (1 ml) was added methanesulfonyl chloride (0.057 ml) at 5°C. After 3 hours at 5°C, the reaction was warmed to ambient temperature and kept for an additional 15 hours. The mixture was diluted with ethyl acetate, washed with saturated NaHCO_3 (2x), water (3x), brine, and dried over MgSO_4 ,
30 filtered, and evaporated. The residue was chromatographed on silica gel (2:1/hexanes:acetone, 1% triethylamine) to yield the pure product (72 mg) as a white solid. Mass spectrometry reveals $[\text{M}+\text{H}^+] = 695$.

35

5 Preparation E: 2'-O-Acetyl-6-O-methyl-3-descladinosyl-3-oxo-10,11-anhydro-14-norerythromycin A

A solution of 2'-O-acetyl-6-O-methyl-3-descladinosyl-3-oxo-11-O-methanesulfonyl-14-norerythromycin A (73 mg) in acetone (1ml) was treated with diazabicycloundecene (32 μ l) at ambient temperature for 18 hours. The mixture was
10 diluted with ethyl acetate, washed with saturated NaHCO_3 , water, brine, and dried over MgSO_4 , filtered, and evaporated. The residue was chromatographed on silica gel (2:1/hexanes:acetone, 1% triethylamine) to yield the pure product (50 mg) as a white solid. Mass spectrometry reveals $[\text{M}+\text{H}]^+ = 598$. ^{13}C -NMR (CDCl_3 , 100 MHz): δ 207.02, 204.50, 169.63, 168.72, 142.52, 139.40, 101.87, 80.61, 80.02, 77.14, 72.66,
15 71.48, 69.09, 63.56, 51.35, 50.56, 47.12, 40.61, 39.73, 37.36, 30.36, 21.32, 21.06, 20.96, 20.67, 18.45, 14.34, 13.89, 13.55, 13.45.

Example 8

20

Preparation A: 2'-O-Benzoyl-6-O-methyl-14,15-dehydroerythromycin A

A solution of 6-O-methyl-14,15-dehydroerythromycin A (668 mg), benzoic anhydride (385 mg), and triethylamine (0.25 mL) in 3.6 mL of CH_2Cl_2 was stirred for 2 days. After addition of saturated NaHCO_3 , the mixture was extracted three times
25 with CH_2Cl_2 . The organic extracts were combined and evaporated to dryness, and the product was purified by silica chromatography (90:9:1 toluene/acetone/ Et_3N) to give 477 mg of product; LC-MS shows $[\text{M}+\text{H}]^+ = 850.6$.

30 Preparation B: 2'-O-Benzoyl-6-O-methyl-4'',11-bis(O-methanesulfonyl)-14,15-dehydroerythromycin A

A solution of 2'-O-benzoyl-6-O-methyl-14,15-dehydroerythromycin A (549 mg) and methanesulfonyl chloride (0.50 mL) in 2.39 mL of pyridine was stirred for 24 hours, then diluted with CH_2Cl_2 and saturated NaHCO_3 . The mixture was extracted three times with CH_2Cl_2 . The organic extracts were combined and evaporated to
35 dryness, and the product was purified by silica chromatography (90:9:1 toluene/acetone/ Et_3N) to give 530 mg of product; LC-MS shows $[\text{M}+\text{H}]^+ = 1006.5$.

5 Preparation C: 2'-O-Benzoyl-6-O-methyl-4''-O-methanesulfonyl-10,11-anhydro-14,15-dehydroerythromycin A

 A mixture of 2'-O-benzoyl-6-O-methyl-4'',11-bis(O-methanesulfonyl)14,15-dehydroerythromycin A (59 mg) and diazabicycloundecene (0.018 mL) in 0.195 mL of acetone was stirred for 24 hours, then dried in vacuo. The product was purified by
10 silica chromatography (90:9:1 toluene/acetone/Et₃N) to give 50 mg of product; LC-MS shows [M+H]⁺ = 910.5.

Preparation D: 2'-O-Benzoyl-6-O-methyl-3-descladinosyl-10,11-anhydro-14,15-dehydroerythromycin A

15 A mixture of 2'-O-benzoyl-6-O-methyl-4''-O-methanesulfonyl-10,11-anhydro-14,15-dehydroerythromycin A (337 mg), 1.5 mL of acetonitrile, and 6.9 mL of 3 N HCl was stirred for 22 hours. The acetonitrile was removed in vacuo, the pH of the aqueous residue was adjusted to 12 by addition of NaOH, and the product was extracted using 4 portions of CH₂Cl₂. The combined extracts were dried and
20 evaporated. The product was purified by silica chromatography (gradient from 96:4 CH₂Cl₂/MeOH to 95:4:1 CH₂Cl₂/MeOH/Et₃N) to give 197 mg, [M+H]⁺ = 674.4.

Preparation E: 2'-O-Benzoyl-6-O-methyl-3-descladinosyl-3-oxo-10,11-anhydro-14,15-dehydroerythromycin A

25 A suspension of 2'-O-benzoyl-6-O-methyl-3-descladinosyl-10,11-anhydro-14,15-dehydroerythromycin A (226 mg) and the Dess-Martin periodinane (427 mg) in 14.6 mL of CH₂Cl₂ (14.6 mL) was stirred for 1 hour. The mixture was diluted with CH₂Cl₂ and saturated NaHCO₃. The product was extracted using 3 portions of CH₂Cl₂, and the extracts were combined, dried, and evaporated. Silica gel
30 chromatography (90:9:1 toluene/acetone/Et₃N) yielded the product, 168 mg. [M+H]⁺ = 672.4. ¹³C-NMR (CDCl₃, 100 MHz): δ 206.78, 203 (br), 168.19, 165.08, 141.36, 139.58, 132.74, 131.51, 130.46, 129.79, 128.25, 120.18, 102.09, 80.79, 80.40, 78.70, 72.52, 71.91, 69.19, 63.76, 51.10, 50.54, 47.08, 40.73, 39.87, 37.77, 31.23, 22.13, 20.98, 18.52, 14.28, 14.15, 13.55.

35

Example 9

5 Preparation A: 6-O-methyl-3-descladinosyl-15-methylerythromycin A

A mixture of 6-O-methyl-15-methylerythromycin A (15.1 g) and 280 mL of 0.5 N HCl was stirred at ambient temperature for 3 hours. The pH was adjusted to 9 by addition of 6 N NaOH, and the resulting precipitate was collected by vacuum filtration, washed with water, and dried. The filtrate was extracted three times with 10 400-mL portions of ethyl acetate. The organic extracts were combined, washed sequentially with saturated NaHCO₃, water, and brine, then dried over MgSO₄, filtered, and evaporated to provide further product. The combined crude products were chromatographed on silica gel to yield 9.35 g of pure 6-O-methyl-3-descladinosyl-15-methylerythromycin A. ES-LC/MS shows [M+H]⁺ = 605.

15 Preparation B: 2'-O-Acetyl-6-O-methyl-3-descladinosyl-15-methylerythromycin A

A solution of acetic anhydride (2.92 mL) in 35 mL of ethyl acetate was added dropwise to a solution of 6-O-methyl-3-descladinosyl-15-methylerythromycin A (9.35 g) in 40 mL of ethyl acetate. The mixture was stirred for 30 minutes after completion 20 of addition, then concentrated. Chromatography on silica gel (2:1 hexanes/acetone) gave 8.35 g of 2'-O-acetyl-6-O-methyl-3-descladinosyl-15-methylerythromycin A. ES-LC/MS shows [M+H]⁺ = 647.

25 Preparation C: 2'-O-Acetyl-6-O-methyl-3-descladinosyl-3-oxo-15-methylerythromycin A

A solution of 2'-O-acetyl-6-O-methyl-3-descladinosyl-15-methylerythromycin A (8.3 g) and 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride (16.51 g) in 64 mL of dichloromethane and 15.47 mL of methylsulfoxide was placed under inert atmosphere and cooled on ice. A solution of pyridinium trifluoroacetate (16.63 g) in 30 64 mL of dichloromethane was added at a rate such that addition would be complete in 4 hours, and the reaction was monitored by thin-layer chromatography. Complete reaction was observed after addition of 73% of the solution, and so the reaction was then quenched by addition of 600 mL of ethyl acetate and 200 mL of saturated NaHCO₃. The organic layer was collected and washed sequentially with saturated 35 NaHCO₃, water, and brine, then dried over MgSO₄, filtered, and evaporated to yield 8.4 g of crude product. Chromatography on silica gel (3:1 hexanes/acetone) gave 6.75

- 5 g of 2'-O-acetyl-6-O-methyl-3-descladinosyl-3-oxo-15-methylerythromycin A. ES-LC/MS shows $[M+H]^+ = 645$.

Preparation D: 2'-O-Acetyl-6-O-methyl-3-descladinosyl-3-oxo-11-O-methanesulfonyl-15-methylerythromycin A

- 10 Methanesulfonylchloride (5.68 mL) was added dropwise to a solution of 2'-O-acetyl-6-O-methyl-3-descladinosyl-3-oxo-15-methylerythromycin A (6.73 g) in 35 mL of pyridine at 0 °C. The mixture was brought to ambient temperature and quenched by addition of 700 mL of ethyl acetate and 200 mL of saturated NaHCO_3 . The organic layer was collected and washed sequentially with saturated NaHCO_3 ,
15 water, and brine, then dried over MgSO_4 , filtered, and evaporated to yield 8.2 g of crude product. Chromatography on silica gel (5:2 hexanes/acetone) gave 5.04 g of 2'-O-acetyl-6-O-methyl-3-descladinosyl-3-oxo-11-O-methanesulfonyl-15-methylerythromycin A. ES-LC/MS shows $[M+H]^+ = 723$.

20 Preparation E: 2'-O-Acetyl-6-O-methyl-3-descladinosyl-3-oxo-10,11-anhydro-15-methylerythromycin A

- 1,8-Diazabicyclo[5.4.0]undec-7-ene (5.22 mL) was added dropwise to a solution of 2'-O-acetyl-6-O-methyl-3-descladinosyl-3-oxo-11-O-methanesulfonyl-15-methylerythromycin A (5.03 g) in 23 mL of acetone. The solution was concentrated
25 after 4.5 hours, and the residue was chromatographed on silica gel (5:2 hexanes/acetone) to give 3.72 g of 2'-O-acetyl-6-O-methyl-3-descladinosyl-3-oxo-10,11-anhydro-15-methylerythromycin A. ES-LC/MS shows $[M+H]^+ = 627$.

Example 10

30 Synthesis of 2'-O-acetyl-6-O-methyl-3-descladinosyl-3-oxo-10,11-anhydro-2-fluoro-15-methyl-erythromycin A

- A solution of 2'-O-acetyl-6-O-methyl-3-descladinosyl-3-oxo-10,11-anhydro-15-methyl-erythromycin A (198 mg, 0.316 mmol) in 2.1 mL of tetrahydrofuran under
35 inert atmosphere was cooled to -78 °C and treated with 0.931 mL of 1.0 M potassium *tert*-butoxide in tetrahydrofuran. The mixture was stirred for 5 minutes, and a solution of N-fluorobenzenesulfonimide (230 mg) in 0.5 mL of tetrahydrofuran was added in three portions over 2 hours. After addition, the reaction was allowed to warm to ambient temperature and kept for an additional 5 hours. Aqueous K_2CO_3 was

5 added, and the mixture was extracted with three 50-mL portions of CH_2Cl_2 . The organic extracts were combined, dried over MgSO_4 , filtered, and evaporated. Chromatography on silica gel (90:9:1 toluene/acetone/ Et_3N) gave 95 mg of product as a white solid. ES-LC/MS: $[\text{M}+\text{H}]^+ = 645$. ^{13}C -NMR (CDCl_3 , 100 MHz): δ 206.95, 203.02 (br), 169.77, 166.08 (d, JCF = 23 Hz), 141.71, 138.43, 101.63, 98.02 (d, JCF = 203 Hz), 80.09 (br), 79.71, 78.27, 73.26, 71.52, 69.08, 63.33, 49.18, 40.61, 40.32, 41.79, 40.61, 40.32, 31.56, 31.47, 30.50, 24.37 (d, JCF = 23 Hz), 23.19, 22.63, 20.95, 20.68, 19.80, 19.47, 14.10, 14.00, 13.55.

Example 11

15 Preparation A: Conversion of Formula (8) to Formula (11) where X = H, R_{13} = propyl and R_2 = allyl.

Step 1: A solution of 2',4''-bis-O-trimethylsilyl-15-methylerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime (7.8 g, 7.44 mmol) in 30 mL of tetrahydrofuran was cooled on ice and treated with 30 mL of methylsulfoxide and 2.58 mL of freshly distilled allyl bromide under inert atmosphere. A mixture of methylsulfoxide (29.8 mL) and 1.0 M potassium *tert*-butoxide in tetrahydrofuran (29.8 mL) was added at a rate of 1.33 molar equivalents of base per hour. The reaction was monitored by thin-layer chromatography (silica gel, 10:1 toluene/acetone), and was judged complete after addition of 3.6 molar equivalents of base. The reaction was diluted with 700 mL of ethyl acetate and washed sequentially with saturated NaHCO_3 , water, and brine. The organic phase was dried with MgSO_4 , filtered, and evaporated to yield 8.08 g of crude 6-O-allyl-2',4''-bis-O-trimethylsilyl-15-methylerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime. This was carried on without further purification.

Step 2: A solution of 6-O-allyl-2',4''-bis-O-trimethylsilyl-15-methylerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime (8.08 g) in 42 mL of acetonitrile was treated with 21 mL of water and 24 mL of acetic acid, and stirred for 18 hours at ambient temperature. The mixture was concentrated after addition of 2-propanol, then repeatedly after addition of toluene to yield 7.7 g of crude product. Chromatography on silica gel (gradient from 2:1 to 1:1 hexanes/acetone + 1% Et_3N) gave 3.75 g of 6-O-allyl-15-methylerythromycin A 9-oxime.

5

Step 3: A solution of 6-O-allyl-15-methylerythromycin A 9-oxime (3.75 g) and sodium hydrosulfite (85%, 5.37 g) in 66 mL of 1:1 ethanol/water was placed under inert atmosphere. Formic acid (0.845 mL) was added dropwise, and the mixture was stirred at 80 °C for 3.5 hours. After cooling to ambient temperature, the reaction was adjusted to pH 10 with 6 N NaOH and extracted three times with 150-mL portions of ethyl acetate. The organic extracts were combined and washed sequentially with saturated NaHCO₃, water, and brine. The organic phase was dried with MgSO₄, filtered, and evaporated to yield 3.42 g of 6-O-allyl-15-methylerythromycin A suitable for further conversion.

15

Preparation B: Conversion of Formula (8) to Formula (11) where X = H, R₁₃ = methyl and R₂ = allyl.

Step 1: A solution of 2',4''-bis-O-trimethylsilyl-14-norerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime (202 mg) in tetrahydrofuran (0.4 mL), DMSO (0.4 mL), and ether (0.04 mL) was cooled to 10 °C and treated with 0.035 mL of freshly distilled allyl bromide under inert atmosphere. A mixture of methylsulfoxide (0.4 mL) and 1.0 M potassium *tert*-butoxide in tetrahydrofuran (0.4 mL) was added at a rate 0.22 mL/hour. The reaction was monitored by thin-layer chromatography (silica gel, 5:1 toluene/acetone. The reaction was diluted with ethyl acetate and washed sequentially with saturated NaHCO₃, water, and brine. The organic phase was dried with MgSO₄, filtered, and evaporated to yield 222 mg of crude 6-O-allyl-2',4''-bis-O-trimethylsilyl-14-norerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime. This was carried on without further purification.

30

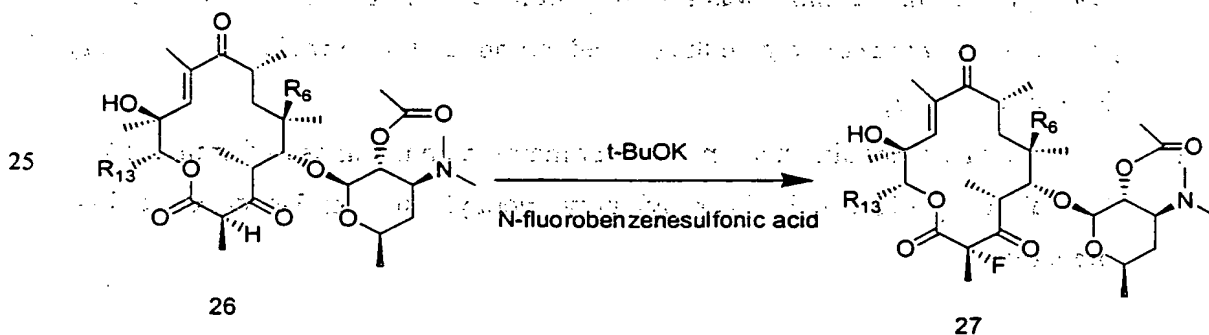
Step 2: A solution of 6-O-allyl-2',4''-bis-O-trimethylsilyl-14-norerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime (222 mg) in 4 mL of acetonitrile was treated with 2 mL of water and 2.4 mL of acetic acid, and stirred for 18 hours at ambient temperature. The mixture was concentrated after addition of 2-propanol, then repeatedly after addition of toluene to yield 220 mg of crude 6-O-allyl-14-norerythromycin A 9-oxime.

35

5 Step 3: A solution of 6-O-allyl-14-norerythromycin A 9-oxime (220 mg) and sodium hydrosulfite (85%, 322 mg) in 4 mL of 1:1 ethanol/water was placed under inert atmosphere. Formic acid (0.050 mL) was added dropwise, and the mixture was stirred at 80 °C for 15 hours. After cooling to ambient temperature, the reaction was adjusted to pH 10 with 6 N NaOH and extracted three times with 150-mL portions of
 10 ethyl acetate. The organic extracts were combined and washed sequentially with saturated NaHCO₃, water, and brine. The organic phase was dried with MgSO₄, filtered, and evaporated to yield 156 mg of 6-O-allyl-14-norerythromycin A suitable for further conversion.

15 Other embodiments: In a similar manner, compounds of formula (11) wherein R_a is allyl, are prepared from an intermediate where R₁₃ is butyl, benzyl, vinyl, or 3-hydroxybutyl.

20

Scheme 5

30

Example 12**Conversion of Formula (11) to Formula (14) (Scheme 1)**

Step 1. A mixture of the compound prepared in Example 11 (77 mg, crude),
 35 0.073 ml of 12 N HCl and water (2 ml) was stirred at ambient temperature for 3 hours. The mixture was brought to pH 8 with 8 N KOH, and extracted with ethyl acetate. The organic extract was washed with brine, dried with MgSO₄, filtered, and

5 evaporated. The residue was chromatographed on silica gel (3:1/hexanes:acetone, 1% triethylamine) to give pure product as a white solid (42 mg).

Step 2. To protect the 2' OH, a mixture the above compound (73 mg), potassium carbonate (20 mg), acetic anhydride (14 μ l) and acetone (1 ml) was stirred at ambient temperature for 18 hours. Ethyl acetate was added, washed with water and
10 brine, dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed on silica gel (3:1/hexanes:acetone, 1% triethylamine) to yield the pure product (71 mg) as a white solid.

Step 3. A solution of the compound resulting from step 2 (99 mg) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) hydrochloride (206 mg) in
15 dichloromethane (2 ml) was treated with DMSO (0.21 ml) and cooled to 5°C. A solution of pyridinium trifluoroacetate (208 mg) in dichloromethane (2 ml) was added via a syringe pump in 4 hours. Ethyl acetate was then added, washed with saturated $NaHCO_3$, water, brine, and dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed on silica gel (3:1/hexanes:acetone, 1% triethylamine) to yield
20 the pure compound of formula (14) (94 mg, R_a is allyl, R_2 is acetate and R_{13} is CH_3).

Step 4. To deprotect 2' OH, a solution of the compound resulting from step 3 (94 mg) in 5 mL methanol was stirred at room temperature for 24 hours. The solvent was removed *in vacuo* to give the desired compound of formula (14) (R_a is allyl, R_2 is H, and R_{13} is CH_3).

25 Other embodiments: In a similar manner, compounds of formula (14) wherein R_a is allyl, R_2 is H, and R_{13} is propyl, butyl, benzyl, vinyl, or 3-hydroxybutyl is prepared.

Example 13

30 Conversions at $-OR_a$

A. Allyl \rightarrow Propyl

A solution of either of the compounds from steps 3 or 4 of Example 12 (0.2 mmol) in ethanol is flushed with nitrogen and 10% palladium on carbon (20 mg) added. The mixture is then flushed with hydrogen and the reaction mixture stirred
35 overnight under positive hydrogen pressure. The reaction mixture is filtered and concentrated *in vacuo* to give a glass. Chromatography on silica gel (95:5:0.5 dichloromethane-methanol-ammonia) gives the propyl compounds as white solids.

5 B. Allyl \rightarrow $-\text{CH}_2\text{CHO}$

Ozone is passed through a -78°C solution in dichloromethane (100 mL) of either of the compounds from steps 3 or 4 of Example 12 (4.0 mmol) for 45 minutes. The reaction mixture is then flushed with nitrogen for 10 minutes. Dimethyl sulfide (1.46 mL, 20 mmol) is added at -78°C and the reaction mixture stirred for 30 minutes at 0°C . The reaction mixture is concentrated *in vacuo* to give a white foam which is used without further purification by heating a solution of the compound in THF (40 mL, 4.0 mmol) and triphenylphosphine (2.62 g, 10.0 mmol) at 55°C for 2.5 hours. The reaction mixture is concentrated *in vacuo* to give a white foam. Chromatography on silica gel (1:1 acetone-hexane, then 75:25:0.5 acetone-hexane-triethylamine) gives the desired compound as a white solid.

C. Allyl \rightarrow $-\text{CH}_2\text{CH}=\text{NOH}$

To a solution in methanol (5 mL) of the compound prepared in B wherein R_3 is $-\text{CH}_2\text{CHO}$, (0.08 mmol) is added triethylamine (31 L, 0.225 mmol) and hydroxylamine hydrochloride (7.7 mg, 0.112 mmol) and the reaction mixture stirred for 6 hours at ambient temperature. The reaction mixture is taken up in ethyl acetate and washed with aqueous 5% sodium bicarbonate and brine, dried over sodium sulfate, and concentrated *in vacuo* to give a clear glass. Chromatography on silica gel (95:5:0.5 dichloromethane-methanol-ammonia) gives the compound as a white solid.

D. $-\text{CH}_2\text{CH}=\text{NOH} \rightarrow -\text{CH}_2\text{CN}$

To a solution under nitrogen of the compound prepared in C (0.267 mmol) in THF (5 mL) is added diisopropylcarbodiimide (83 μL , 0.534 mmol) and CuCl (2.7 mg, 0.027 mmol) and the reaction mixture is stirred overnight at ambient temperature. The reaction mixture is taken up in ethyl acetate and washed with aqueous 5% sodium bicarbonate and brine, dried over sodium sulfate, and concentrated *in vacuo* to give a clear glass. Chromatography on silica gel (95:5:0.5 dichloromethane-methanol-ammonia) gives the desired compound as a white solid.

E. $-\text{CH}_2\text{CHO} \rightarrow -\text{CH}_2\text{CH}_2\text{NH}_2$

To a solution in methanol (10 mL) of the compound prepared in B (0.276 mmol) is added ammonium acetate (212 mg, 2.76 mmol) and the mixture is cooled to 0°C . Sodium cyanoborohydride (34 mg, 0.553 mmol) is added and the reaction mixture stirred for 30 hours at 0°C . The reaction mixture is taken up in ethyl acetate

5 and washed with aqueous 5% sodium carbonate, aqueous 2% tris(hydroxymethyl)aminomethane, and brine, dried over sodium sulfate, filtered, and concentrated *in vacuo*. Chromatography on silica gel (90:10:0.5 dichloromethane-methanol-ammonia) gives the desired compound as a white solid.

F. $-\text{CH}_2\text{CHO} \rightarrow -\text{CH}_2\text{CH}_2\text{NHCH}_2\text{-Phenyl}$

10 To a 0°C solution in methanol (10 mL) of the compound prepared in B (0.200 mmol) is added acetic acid (114 µL, 2.00 mmol) and benzylamine (218 µL, 2.00 mmol) and the mixture is stirred for 10 minutes. Sodium cyanoborohydride (24.8 mg, 0.400 mmol) is added and the reaction mixture stirred for 16 hours. Additional sodium cyanoborohydride (24.8 mg, 0.400 mmol) is then added and stirring continued
15 for 5 hours. The reaction mixture is taken up in ethyl acetate and washed with aqueous 5% sodium carbonate, aqueous 2% tris(hydroxymethyl)aminomethane, and brine, dried over sodium sulfate, filtered, and concentrated *in vacuo*. Chromatography on silica gel (95:5:0.5 dichloromethane-methanol-ammonia) followed by a second chromatography (50:50:0.5 acetone-hexanes-triethylamine) gives the desired
20 compound as a white foam.

G. $-\text{CH}_2\text{CHO} \rightarrow -\text{CH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2\text{-Phenyl}$

To a 0°C solution in methanol (10 mL) of the compound prepared in B (0.200 mmol) is added acetic acid (114 µL, 2.00 mmol) and phenethylamine (218 L, 2.00 mmol) and the mixture stirred for 10 minutes. Sodium cyanoborohydride (24.8 mg, 0.400 mmol) is added and the reaction mixture stirred for 16 hours. The reaction
25 mixture is taken up in ethyl acetate and washed with aqueous 5% sodium carbonate, aqueous 2% tris(hydroxymethyl)aminomethane, and brine, dried over sodium sulfate, filtered, and concentrated *in vacuo*. Chromatography on silica gel (90:10:0.5 dichloromethane-methanol-ammonia) gives the desired compound.

30 H. $-\text{CH}_2\text{CHO} \rightarrow -\text{CH}_2\text{CH}_2\text{NHCH}(\text{CO}_2\text{CH}_3)\text{CH}_2\text{-Phenyl}$

To a 0°C solution in methanol (10 mL) of the compound prepared in B (0.200 mmol) is added L-phenylalanine methyl ester hydrochloride (129 mg, 0.600 mmol) and the mixture stirred for 10 minutes. Sodium cyanoborohydride 924.8 mg, 0.400 mmol) is added and the reaction mixture stirred for 22 hours. The reaction mixture is
35 taken up in ethyl acetate and washed with aqueous 5% sodium carbonate, aqueous 2% tris(hydroxymethyl)aminomethane, and brine, dried over sodium sulfate, filtered, and

5 concentrated *in vacuo*. Chromatography on silica gel (95:5:0.5 dichloromethane-methanol-ammonia) gives the desired compound.

I. $-\text{CH}_2\text{CHO} \rightarrow -\text{CH}_2\text{CH}_2\text{NHCH}_2-(4\text{-pyridyl})$

The desired compound is prepared according to the method in G, except substituting 4-aminomethylpyridine for phenethylamine.

10 J. $-\text{CH}_2\text{CH}_2\text{NH}_2 \rightarrow -\text{CH}_2\text{CH}_2\text{NHCH}_2-(4\text{-quinolyl})$

To a solution of the compound prepared in E (0.15 mmol) in methanol (2 mL) is added 4-quinolinecarboxaldehyde (23 mg, 0.15 mmol), acetic acid (8.6 L, 0.15 mmol), and sodium cyanoborohydride (9.4 mg, 0.15 mmol) and the reaction mixture is stirred for 15 hours. The reaction mixture is taken up in ethyl acetate and washed
15 with aqueous 5% sodium carbonate, aqueous 2% tris(hydroxymethyl)aminomethane, and brine, dried over sodium sulfate, filtered, and concentrated *in vacuo*. Chromatography on silica gel (95:10:0.5 dichloromethane-methanol-ammonia) gives the desired compound.

K. Allyl $\rightarrow -\text{CH}_2\text{CH}=\text{CH}-\text{Phenyl}$

20 To a solution under nitrogen of the 2' protected compound prepared in Example 12 (1.00 mmol), palladium(II)acetate (22 mg, 0.100 mmol), and triphenylphosphine (52 mg, 0.200 mmol) in acetonitrile (5 mL) was added iodobenzene (220 μL , 2.00 mmol) and triethylamine (280 μL , 2.00 mmol) and the mixture is cooled to -78°C , degassed, and sealed. The reaction mixture is then
25 warmed to 60°C for 0.5 hours and stirred at 80°C for 12 hours, taken up in ethyl acetate and washed twice with aqueous 5% sodium bicarbonate, once with aqueous 2% tris(hydroxymethyl)aminomethane, and once with brine, dried over sodium sulfate, filtered, and concentrated *in vacuo*. Chromatography on silica gel (95:5:0.5 dichloromethane-methanol-ammonia) gives the desired compound.

30 Deprotection is accomplished by heating in methanol.

Other embodiments of formula (14) where R_2 is H and R_{13} is propyl, butyl, benzyl, vinyl, or 3-hydroxybutyl are those wherein R_4 is:

$-\text{CH}_2\text{CH}_2\text{CH}_2\text{-phenyl};$	$-\text{CH}_2\text{CH}=\text{CH}-(4\text{-methoxyphenyl});$
$-\text{CH}_2\text{CH}=\text{CH}-(4\text{-chlorophenyl});$	$-\text{CH}_2\text{CH}=\text{CH}-(3\text{-quinolyl});$
$-\text{CH}_2\text{CH}_2\text{CH}_2\text{OH};$	$-\text{CH}_2\text{C}(\text{O})\text{OH};$
$-\text{CH}_2\text{CH}_2\text{NHCH}_3;$	$-\text{CH}_2\text{CH}_2\text{NHCH}_2\text{OH};$
$-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_2;$	$-\text{CH}_2\text{CH}_2(1\text{-morpholinyl});$

-CH ₂ C(O)NH ₂ ;	-CH ₂ NHC(O)NH ₂ ;
-CH ₂ NHC(O)CH ₃ ;	-CH ₂ F;
-CH ₂ CH ₂ OCH ₃ ;	-CH ₂ CH ₃ ;
-CH ₂ CH=CH(CH ₃) ₂ ;	-CH ₂ CH ₂ CH(CH ₃)CH ₃ ;
-CH ₂ CH ₂ OCH ₂ CH ₂ OCH ₃ ;	-CH ₂ SCH ₃ ;
-cyclopropyl;	-CH ₂ OCH ₃ ;
-CH ₂ CH ₂ F;	-CH ₂ -cyclopropyl;
-CH ₂ CH ₂ CHO;	-C(O)CH ₂ CH ₂ CH ₃ ;
-CH ₂ -(4-nitrophenyl);	-CH ₂ -(4-chlorophenyl);
-CH ₂ -(4-methoxyphenyl);	-CH ₂ -(4-cyanophenyl);
-CH ₂ CH=CHC(O)OCH ₃ ;	-CH ₂ CH=CHC(O)OCH ₂ CH ₃ ;
-CH ₂ CH=CHCH ₃ ;	-CH ₂ CH=CHCH ₂ CH ₃ ;
-CH ₂ CH=CHCH ₂ CH ₂ CH ₃ ;	-CH ₂ CH=CHSO ₂ -phenyl;
-CH ₂ CCSi(CH ₃) ₃ ;	-CH ₂ CCCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃ ;
-CH ₂ C CCH ₃ ;	-CH ₂ -(2-pyridyl);
-CH ₂ -(3-pyridyl);	-CH ₂ -(4-pyridyl);
-CH ₂ -(4-quinolyl);	-CH ₂ NO ₂ ;
-CH ₂ C(O)OCH ₃ ;	-CH ₂ C(O)-phenyl;
-CH ₂ C(O)CH ₂ CH ₃ ;	-CH ₂ Cl;
-CH ₂ S(O) ₂ -phenyl;	-CH ₂ CH=CHBr;
-CH ₂ CH=CH-(4-quinolyl);	-CH ₂ CH ₂ CH ₂ -(4-quinolyl);
-CH ₂ CH=CH-(5-quinolyl);	-CH ₂ CH ₂ CH ₂ -(5-quinolyl);
-CH ₂ CH=CH-(4-benzoxazolyl); or	-CH ₂ CH=CH-(7-benzimidazolyl);

Example 14

Preparation of Compound of Formula (I) where R = H, R₂ = H, X = H, R₆ = -OR₃, R₃ is -CH₂CH=CH₂ (Scheme 3)

Step 1: Preparation to form 10,11-anhydro form of intermediate compound (20): R₂ is -CH₂CH=CH₂, R₃ is benzoyl.

A: 6-O-allyl-3-descladinosyl-15-methyl-erythromycin A

A mixture of 6-O-allyl-15-methylerythromycin A (6.58 g) and 125 mL of 0.5 N HCl was stirred at ambient temperature for 20 hours. The pH was adjusted to 10 by addition of 6 N NaOH, and the mixture was extracted three times with 225-mL

5 portions of ethyl acetate. The organic extracts were combined, washed sequentially with saturated NaHCO_3 , water, and brine, then dried over MgSO_4 , filtered, and evaporated. The crude product was chromatographed on silica gel (3:2 toluene/acetone + 1% Et_3N) to yield 3.04 g of pure 6-O-allyl-3-descladinosyl-15-methylerythromycin A. ES-LC/MS shows $[\text{M}+\text{H}]^+ = 617$.

10

B. 2'-O-Benzoyl-6-O-allyl-3-descladinosyl-15-methyl-erythromycin A

6-O-Allyl-3-descladinosyl-15-methylerythromycin A (2.43 g, 3.86 mmol, 1.00 eq) and benzoic anhydride (1.78 g, 7.72 mmol, 2.00 eq) were placed in a round-bottomed flask and flushed with N_2 . Ethyl acetate (17.5 mL) was added. The solution
15 was stirred for 3.5 h and then diluted with 400 mL of EtOAc and washed twice with 150 mL of saturated aqueous NaHCO_3 and once each with 150 mL of water and brine. The organic phase was dried over MgSO_4 , filtered, and concentrated. Purification by flash chromatography over silica gel (3:1 hexanes:acetone + 1% Et_3N) gave 1.94 g (68.1%) of the desired product as a white solid. ES-LC/MS shows $[\text{M}+\text{H}]^+ = 721$. ^{13}C
20 NMR (100.6 MHz, CDCl_3) δ 219.4, 174.3, 165.4, 135.3, 132.6, 130.8, 129.7, 128.2, 117.2, 99.7, 80.7, 79.0, 77.9, 77.7, 75.1, 74.3, 72.3, 69.0, 64.7, 63.3, 45.6, 43.9, 40.7, 37.9, 37.7, 35.7, 32.1, 30.8, 21.1, 20.2, 19.3, 18.1, 16.3, 15.1, 14.0, 12.4, 7.7.

C. 2'-O-Benzoyl-6-O-allyl-3-descladinosyl-3-oxo-15-methyl-erythromycin A

25 N-Chlorosuccinimide (0.510 g, 3.82 mmol, 1.50 eq) was dissolved in 13 mL of anhydrous CH_2Cl_2 and cooled to -10°C under N_2 . Methyl sulfide (0.328 mL, 4.46 mmol, 1.75 eq) was added, and the reaction was stirred for 15 min. A solution of 2'-O-benzoyl-6-O-allyl-3-descladinosyl-15-methylerythromycin A (1.87 g, 2.55 mmol, 1.00 eq) in 13 mL of anhydrous CH_2Cl_2 was added dropwise. After 30 min, freshly
30 distilled Et_3N (0.355 mL, 2.55 mmol, 1.00 eq) was added; and the reaction was brought up to 0°C over 30 min. The reaction mixture was diluted with 400 mL EtOAc and washed successively with 100 mL each of saturated aqueous NaHCO_3 , water, and brine. The organic layer was dried over MgSO_4 , filtered, concentrated, and purified by flash chromatography (9:1 hexanes:acetone + 1% Et_3N) to give 0.931 g
35 (49.9%) of the desired product as a white solid. ES-LC/MS shows $[\text{M}+\text{H}]^+ = 719$. ^{13}C NMR (100.6 MHz, CDCl_3) δ 219.1, 206.1, 169.5, 165.3, 135.3, 132.7, 129.0, 129.7,

5 128.3, 117.4, 100.7, 78.5, 76.6, 75.3, 74.2, 72.1, 69.2, 69.0, 64.5, 63.7, 50.6, 45.3, 44.8, 40.7, 38.3, 37.8, 31.7, 31.0, 21.1, 20.2, 19.5, 18.1, 16.5, 14.5, 14.0, 12.6, 12.2.

D. 2'-O-Benzoyl-6-O-allyl-3-descladinosyl-3-oxo-11-O-methanesulfonyl-15-methyl-erythromycin A

10 2'-O-Benzoyl-6-O-Allyl-3-descladinosyl-3-oxo-15-methylerythromycin A (904 mg, 1.24 mmol, 1.00 eq) was dissolved in freshly distilled pyridine (4 mL) and cooled to 0 °C. Methanesulfonyl chloride (0.478 mL, 6.17 mmol, 5.00 eq) was added dropwise. The reaction was allowed to come to ambient temperature and stirred overnight. The mixture was diluted with 350 mL of EtOAc and quenched with 100
15 mL of saturated aqueous NaHCO₃. The layers were separated, and the organic phase was washed successively with 100 mL each of water and brine. The organic phase was dried over MgSO₄, filtered, and concentrated. Flash chromatography over silica gel (4:1 hexanes:acetone + 1% Et₃N) gave 741 mg (74.1%) of the desired compound as a white solid. ¹³C NMR (100.6 MHz, CDCl₃) δ 203.0, 168.9, 165.0, 137.6, 133.1,
20 130.3, 129.8, 128.5, 114.4, 108.8, 102.2, 91.1, 84.4, 81.6, 78.8, 72.2, 69.2, 64.3, 63.9, 52.1, 46.6, 45.8, 40.7, 38.8, 38.2, 35.9, 31.8, 30.9, 29.7, 24.8, 21.0, 19.6, 18.2, 15.5, 15.4, 13.8, 13.5.

E. 2'-O-Benzoyl-6-O-allyl-3-descladinosyl-3-oxo-10,11-anhydro-15-methyl-erythromycin A

25 2'-O-Benzoyl-6-O-allyl-3-descladinosyl-3-oxo-11-methanesulfonyl-15-methyl-erythromycin A (705 mg, 0.870 mmol, 1.00 eq) was dissolved in acetone (3 mL), and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.651 mL, 4.35 mmol, 5.00 eq) was added dropwise. The reaction was stirred at ambient temperature for 6 h and then
30 concentrated. Flash chromatography over silica gel (4:1 hexanes:acetone + 1% Et₃N) gave 486 mg (78.0%) of the desired compound as a white solid. ¹³C NMR (100.6 MHz, CDCl₃) δ 210.1, 208.4, 170.2, 165.2, 141.0, 140.2, 136.3, 132.7, 130.4, 129.8, 128.2, 115.5, 100.6, 81.0, 78.7, 77.2, 73.8, 72.0, 69.1, 64.6, 63.3, 51.0, 47.4, 40.8, 39.4, 36.2, 31.9, 31.3, 23.6, 21.2, 21.1, 21.0, 19.4, 14.1, 13.9, 13.7, 13.1.

35

Step 2: Derivatization of Position 12 hydroxyl of compound (18) from Illustrative

Scheme 3: R₁ is -CH₂CH=CH₂, R₂ is benzoyl.

5 2'-O-Benzoyl-6-O-allyl-10,11-anhydro-3-descladinosyl-3-oxo-15-methyl-erythromycin A (227 mg, 0.317 mmol, 1.00 eq) was dissolved in 1.3 mL of freshly distilled THF and cooled to -15 °C under N₂. Sodium hydride (25 mg of a 60% dispersion in mineral oil, 0.634 mmol, 2.00 eq) was added, and the reaction was stirred for 15 min. A solution of 1,1-carbonyldiimidazole (140 mg, 0.866 mmol, 3.00
10 eq) in 1.3 mL of freshly distilled THF was added dropwise. After stirring for 30 min, the reaction was allowed to warm to ambient temperature over 1.5 h. The mixture was diluted with 100 mL of EtOAc and washed successively with 30 mL each of saturated aqueous NaHCO₃, water, and brine. The organic phase was dried over MgSO₄, filtered, and concentrated to give 275 mg of crude product (100%) which was
15 dissolved in 2 mL of ACN and 0.2 mL of anhydrous THF. Saturated aqueous ammonium hydroxide (2 mL) was added. The reaction was sealed and stirred for 2 d. Volatiles were removed under reduced pressure, and the residue was re-dissolved in 100 mL of EtOAc. The solution was washed successively with 30 mL each of saturated aqueous NaHCO₃, water, and brine. The organic phase was dried over
20 MgSO₄, filtered, and concentrated. Flash chromatography of the crude product (4:1 hexanes:acetone + 1% Et₃N) yielded 184 mg (76.5%) of the desired product.

Example 15

25 Preparation A: formula (I): X = H, R₂ is -CH₂-CH=CH-(3-quinolyl)
Step 1: 2'-O-Benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate (40 mg, 0.0528 mmol, 1.0 eq), tris(dibenzylideneacetone) dipalladium(0)-chloroform adduct (14 mg, 0.014 mmol, 0.5 eq), tri-*o*-tolylphosphine (17 mg, 0.055 mmol, 1.0 eq), and 3-bromoquinoline (72
30 µl, 0.53 mmol, 10 eq) were placed in a round-bottom flask which was flushed with N₂. Degassed acetonitrile (1 mL) and freshly distilled Et₃N (0.015 mL, 0.11 mmol, 2.0 eq) were added. The reaction was refluxed for 63 h. The mixture was returned to ambient temperature and diluted with 40 mL of EtOAc. The solution was washed successively with 10 mL each of saturated aqueous NaHCO₃, water, and brine. The
35 organic phase was dried over MgSO₄, filtered, and concentrated. Flash chromatography of the crude product (gradient from 5:1 to 2:1 hexanes:acetone + 1% Et₃N) yielded 34 mg of the desired product.

5

Step 2: The above product (34 mg) was dissolved in 1 mL of methanol, sealed, and refluxed at 80 °C for 16 h. Volatiles were removed under reduced pressure. Flash chromatography (1:1 hexanes:acetone + 1% Et₃N) gave the desired product as a light yellow solid (25 mg, 61% over two steps). ES-LC/MS: [M+H]⁺ = 780.5. ¹³C-NMR (CDCl₃, 100 MHz): δ 217.44, 205.37, 169.48, 157.69, 149.71, 147.61, 132.51, 129.96, 129.56, 129.15, 129.05, 128.49, 128.05, 126.70, 102.90, 83.42, 78.71, 76.42, 75.91, 70.22, 69.53, 65.83, 64.31, 58.12, 50.81, 46.29, 46.12, 45.05, 40.18 (2 C), 39.05, 37.31, 31.64, 28.19, 21.15, 20.18, 19.43, 18.05, 14.38, 14.11, 13.76, 13.63 (2 C).

15 Preparation B: formula (I): X = H, R₂ is -CH₂-CH=CH-(3-(6-fluoroquinolyl))

This was prepared according to the method of Preparation A using 3-bromo-6-fluoroquinoline in place of 3-bromoquinoline. ES-LC/MS: [M+H]⁺ = 798.5. ¹³C-NMR (CDCl₃, 100 MHz): δ 217.49, 205.36, 169.54, 160.6 (J_{CF} = 248 Hz), 157.68, 149.05, 144.69, 131.84, 131.64 (J_{CF} = 9 Hz), 130.28, 129.63, 129.31, 128.7 (J_{CF} = 10 Hz), 119.20 (J_{CF} = 27 Hz), 110.87 (J_{CF} = 22 Hz), 102.94, 83.42, 78.77, 76.44, 75.91, 70.22, 69.55, 65.84, 64.24, 58.09, 50.83, 46.36, 46.06, 45.05, 40.18 (2 C) 39.04, 37.32, 31.63, 28.19, 21.16, 20.19, 19.46, 18.04, 14.37, 14.18, 13.76, 13.62 (2 C).

Preparation C: formula (I): X = H, R₂ is -CH₂-CH=CH-(3-(6-chloroquinolyl))

25 This is prepared according to the method of Preparation A using 3-bromo-6-chloroquinoline in place of 3-bromoquinoline. ES-LC/MS: [M+H]⁺ = 814.5. ¹³C-NMR (CDCl₃, 100 MHz): δ 217.48, 205.35, 169.55, 157.67, 149.90, 145.92, 132.42, 131.49, 130.80, 130.44, 129.92, 129.49, 129.46, 128.71, 126.57, 102.94, 83.41, 78.78, 76.45, 75.91, 70.22, 69.54, 65.83, 64.23, 58.07, 50.83, 46.39, 45.99, 45.04, 40.17 (2 C), 39.03, 37.32, 31.62, 31.53, 28.18, 21.16, 20.17, 19.49, 18.04, 14.36, 14.21, 13.76, 13.61 (2 C).

Preparation D: formula (I): X = H, R₂ is -CH₂-CH=CH-(4-isoquinolyl)

35 This was prepared according to the method of Preparation A using 4-bromoisquinoline in place of 3-bromoquinoline. ES-LC/MS: [M+H]⁺ = 781. ¹³C-NMR (CDCl₃, 100 MHz): δ 217.19, 205.43, 169.75, 157.39, 152.07, 140.74, 133.61,

5 130.65, 130.44, 128.07, 127.72, 127.05, 126.89, 122.77, 102.85, 83.28, 78.74, 75.72,
70.22, 69.51, 65.88, 64.45, 58.10, 50.91, 46.07, 45.09, 40.18 (2 C) 38.99, 37.34,
31.48, 29.66, 28.28, 21.18, 20.39, 19.33, 14.53, 14.01, 13.86, 13.66, 13.62.

Preparation E: formula (I): X = H, R₂ is -CH₂-CH=CH-(3-pyridyl)

10 This was prepared according to the method of Preparation A using 3-bromopyridine in place of 3-bromoquinoline. LC/MS: [M+H]⁺ = 731. ¹³C-NMR (CDCl₃, 100 MHz): δ 217.39, 205.27, 169.50, 157.61, 148.81, 148.68, 132.63, 132.16, 129.65, 128.18, 123.46, 102.91, 83.36, 78.63, 76.35, 75.79, 70.20, 69.52, 65.83, 64.17, 58.06, 50.78, 46.28, 45.03, 40.16 (2 C), 38.96, 37.29, 31.64, 31.52, 28.19,
15 22.58, 21.14, 20.21, 19.42, 18.04, 1.35, 14.12, 14.05, 13.79, 13.61 (2 C).

Preparation F: formula (I): X = H, R₂ is -CH₂-CH=CH-(3-(6-methylquinolyl))

This was prepared according to the method of Preparation A using 3-bromo-6-methylquinoline in place of 3-bromoquinoline. ES-LC/MS: [M+H]⁺ = 795. ¹³C-NMR
20 (CDCl₃, 100 MHz): δ 217.37, 205.35, 169.47, 157.65, 148.82, 146.23, 136.45, 131.87, 131.37, 130.09, 129.51, 128.78, 128.22, 128.06, 126.86, 102.87, 83.40, 78.68, 75.91, 70.20, 69.47, 65.83, 64.33, 58.11, 50.81, 46.28, 45.04, 40.15 (2 C), 39.05, 37.31, 31.64, 28.24, 21.52, 21.14, 20.18, 19.45, 18.05, 14.38, 14.11, 13.77, 13.63 (2 C).

25 Preparation G: formula (I): X = H, R₂ is -CH₂-CH=CH-(3-(6-aminoquinolyl))

This was prepared according to the method of Preparation A using 3-bromo-6-aminoquinoline in place of 3-bromoquinoline. ES-LC/MS: [M+H]⁺ = 796.

Preparation H: formula (I): X = H, R₂ is -CH₂-CH=CH-(3-(5-isoxazol-3-yl)thienyl)

30 This is prepared according to the method of Preparation A, using 5-(isoxazol-3-yl)-2-bromothiophene in place of 3-bromoquinoline.

Preparation I: formula (I): X = H, R₂ is -CH₂-CH=CH-(6-quinolyl)

This is prepared according to the method of Preparation A, using 6-bromoquinoline in
35 place of 3-bromoquinoline.

Preparation J: formula (I): X = H, R₂ is -CH₂-CH=CH-(3-quinoxal-6-yl)

5 This is prepared according to the method of Preparation A using 6-bromoquinoxaline in place of 3-bromoquinoline.

Preparation K: formula (I): X = H, R₂ is -CH₂-CH=CH-(5-(N-(2-pyridyl)-2-furamidyl)

10 This is prepared according to the method of Preparation A, using N-(2-pyridyl) 5-bromo-2-furamide in place of 3-bromoquinoline.

Preparation AA: formula (I): X = F, R₂ is -CH₂-CH=CH-(3-quinolyl)

This was prepared according to the method of Preparation A using 2'-O-
15 benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate. LC/MS: [M+H]⁺ = 798.6, ¹⁹F-NMR (CDCl₃, 376 MHz): δ -163.93. ¹³C-NMR (CDCl₃, 100 MHz): δ 217.97, 204.28 (J_{CF} = 27 Hz), 165.62 (J_{CF} = 23 Hz),
20 157.18, 149.71, 147.70, 132.65, 130.25, 129.53, 129.22, 129.12, 129.06, 128.15, 128.08, 126.78, 104.10, 98.02 (J_{CF} = 206 Hz), 83.40, 79.59, 79.37, 77.57, 70.41, 69.74, 65.85, 64.36, 58.11, 44.23, 40.83 (J_{CF} = 1.5 Hz), 40.25 (2 C), 39.04, 37.45, 31.37, 28.16, 25.30 (J_{CF} = 22 Hz), 21.19, 20.86, 19.54, 17.67, 15.46 (J_{CF} = 1.7 Hz),, 13.82, 13.80, 13.29.

25 Preparation BB: formula (I): X = F, R₂ is -CH₂-CH=CH-(3-(6-fluoroquinolyl)

This is prepared according to the method of Preparation B using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.
30

Preparation CC: formula (I): X = F, R₂ is -CH₂-CH=CH-(3-(6-chloroquinolyl)

This is prepared according to the method of Preparation C using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.
35

5 Preparation DD: formula (I): X = F, R₂ is -CH₂-CH=CH-(4-isoquinolyl)

This is prepared according to the method of Preparation D using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

Preparation EE: formula (I): X = F, R₂ is -CH₂-CH=CH-(3-pyridyl)

This is prepared according to the method of Preparation E using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

Preparation FF: formula (I): X = F, R₂ is -CH₂-CH=CH-(3-(6-methylquinolyl))

This is prepared according to the method of Preparation F using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

Preparation GG: formula (I): X = F, R₂ is -CH₂-CH=CH-(3-(6-aminoquinolyl))

This is prepared according to the method of Preparation G using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

Preparation HH: formula (I): X = F, R₂ is -CH₂-CH=CH-(3-(5-isoxazol-3-yl)thienyl)

This is prepared according to the method of Preparation H using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

5 Preparation II: formula (I): X = F, R₂ is -CH₂-CH=CH-(6-quinolyl)

This is prepared according to the method of Preparation I using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

10

Preparation JJ: formula (I): X = F, R₂ is -CH₂-CH=CH-(3-quinoxal-6-yl)

This is prepared according to the method of Preparation J using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

15

Preparation KK: formula (I): X = F, R₂ is -CH₂-CH=CH-(5-(N-(2-pyridyl)-2-furamidyl)

This is prepared according to the method of Preparation K using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

25

Example 16

Preparation of Compound of Formula (I): X = H, R₂ is -O-propargyl (Scheme 3)

Step 1: A solution of 2',4''-bis-O-trimethylsilyl-13-methylerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime (100 mg) in 0.1 mL of tetrahydrofuran, 0.1 mL of ether, and 0.1 mL of DMSO was cooled to 10 °C and treated with 0.028 mL of 3-bromo-1-(trimethylsilyl)-1-propyne under inert atmosphere. A mixture of methylsulfoxide (0.19 mL) and 1.0 M potassium *tert*-butoxide in tetrahydrofuran (0.38 mL) was added at a rate of 2.0 molar equivalents of base per hour. Additional equivalents (0.014 mL) of the TMS-propargyl bromide were added after 0.5 and 1 hours. The reaction was monitored by thin-layer chromatography (silica gel, 10:1 toluene/acetone), and was judged complete after addition of 2.3 molar equivalents of

35

5 base. The reaction was diluted with 100 mL of ethyl acetate and 30 mL of saturated NaHCO_3 , and washed sequentially with saturated NaHCO_3 , water, and brine. The organic phase was dried with MgSO_4 , filtered, and evaporated. The crude product was chromatographed on silica gel (40:1 hexanes/acetone + 1% Et_3N) to yield partially purified 6-O-(3-trimethylsilyl)propargyl-2',4''-bis-O-trimethylsilyl-15-
10 methylerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime.

Step 2: A solution of the impure 6-O-(3-trimethylsilyl)propargyl-2',4''-bis-O-trimethylsilyl-15-methylerythromycin A 9-[O-(1-isopropoxycyclohexyl)]oxime from above (0.88 g) in 4.4 mL of acetonitrile is treated with 2.2 mL of water and 2.5 mL of
15 acetic acid, and stirred for 24 hours at ambient temperature. The mixture is concentrated after addition of 2-propanol, then repeatedly after addition of toluene. This material is stirred with potassium carbonate and methanol (6 mL) for 2.5 hours. The mixture is diluted with ethyl acetate (200 mL), and washed sequentially with saturated NaHCO_3 , water, and brine. The organic phase is dried with MgSO_4 , filtered,
20 and evaporated to yield the product.

Step 3: A solution of the product from (iii) and sodium hydrosulfite (0.59 g) in 7 mL of 1:1 ethanol/water is placed under inert atmosphere. Formic acid (0.096 mL) is added dropwise, and the mixture is stirred at 80 °C for 5 hours. After cooling to
25 ambient temperature, the reaction is adjusted to pH 10 with 6 N NaOH and extracted three times with 150-mL portions of ethyl acetate. The organic extracts are combined and washed sequentially with saturated NaHCO_3 , water, and brine. The organic phase is dried with MgSO_4 , filtered, and evaporated to yield 6-O-propargyl-15-methylerythromycin A suitable for further conversion. Pure material can be prepared
30 by chromatography on silica gel.

Step 4: A mixture of 6-O-propargyl-15-methylerythromycin A (0.40 g) and 6 mL of 0.6 N HCl is stirred at ambient temperature for 17 hours. The pH is adjusted to 9 by addition of 6 N NaOH, and 150 mL of ethyl acetate is added. The organic extracts are
35 washed sequentially with saturated NaHCO_3 , water, and brine, then dried over MgSO_4 , filtered, and evaporated to provide further product. The crude product is

5 chromatographed on silica gel to give pure 6-O-propargyl-3-descladinosyl-15-methylerythromycin A.

Step 5: A solution of 6-O-propargyl-3-descladinosyl-15-methylerythromycin A (0.16 g) and benzoic anhydride (0.12 g) in 1.3 mL of ethyl acetate is stirred for 17 h, then
10 washed sequentially with saturated NaHCO_3 , water, and brine. The solution is dried over MgSO_4 , filtered, and evaporated. The crude product is chromatographed on silica gel to yield 2'-O-benzoyl-6-O-propargyl-3-descladinosyl-15-methylerythromycin A.

Step 6: N-Chlorosuccinimide (0.510 g, 3.82 mmol, 1.50 eq) is dissolved in 13 mL of anhydrous CH_2Cl_2 and cooled to -10°C under N_2 . Methyl sulfide (0.328 mL, 4.46 mmol, 1.75 eq) is added, and the reaction is stirred for 15 min. A solution of 2'-O-benzoyl-6-O-propargyl-3-descladinosyl-15-methylerythromycin A (1.87 g, 2.55 mmol, 1.00 eq) in 13 mL of anhydrous CH_2Cl_2 is added dropwise. After 30 min,
20 freshly distilled Et_3N (0.355 mL, 2.55 mmol, 1.00 eq) is added, and the reaction is brought up to 0°C over 30 min. The reaction mixture is diluted with 400 mL EtOAc and washed successively with 100 mL each of saturated aqueous NaHCO_3 , water, and brine. The organic layer is dried over MgSO_4 , filtered, concentrated, and purified by chromatography.

Step 7: 2'-O-Benzoyl-6-O-propargyl-3-descladinosyl-3-oxo-15-methylerythromycin A (904 mg) is dissolved in freshly distilled pyridine (4 mL) and cooled to 0°C .

Methanesulfonyl chloride (0.478 mL, 6.17 mmol, 5.00 eq) is added dropwise. The reaction is allowed to come to ambient temperature and stirred overnight. The
30 mixture is diluted with 350 mL of EtOAc and quenched with 100 mL of saturated aqueous NaHCO_3 . The layers are separated, and the organic phase is washed successively with 100 mL each of water and brine. The organic phase is dried over MgSO_4 , filtered, and concentrated. Flash chromatography over silica gel yields the product.

Step 8: 2'-O-Benzoyl-6-O-propargyl-3-descladinosyl-3-oxo-11-methanesulfonyl-15-methyl-erythromycin A (705 mg) is dissolved in acetone (3 mL), and 1,8-

5 diazabicyclo[5.4.0]-undec-7-ene (0.651 mL, 4.35 mmol, 5.00 eq) is added dropwise. The reaction is stirred at ambient temperature for 6 h and then concentrated. Flash chromatography over silica gel yields the product.

Step 9: 2'-O-Benzoyl-6-O-propargyl-10,11-anhydro-3-descladinosyl-3-oxo-15-
10 methylerythromycin A (227 mg) is dissolved in 1.3 mL of freshly distilled THF and cooled to -15 °C under N₂. Sodium hydride (25 mg of a 60% dispersion in mineral oil, 0.634 mmol, 2.00 eq) is added, and the reaction was stirred for 15 min. A solution of 1,1-carbonyldiimidazole (140 mg) in 1.3 mL of freshly distilled THF is added dropwise. After stirring for 30 min, the reaction is allowed to warm to ambient
15 temperature over 1.5 h. The mixture is diluted with 100 mL of EtOAc and washed successively with 30 mL each of saturated aqueous NaHCO₃, water, and brine. The organic phase is dried over MgSO₄, filtered, and concentrated, then the residue is dissolved in 2 mL of ACN and 0.2 mL of anhydrous THF. Saturated aqueous ammonium hydroxide (2 mL) is added. The reaction is sealed and stirred for 2 days.
20 Volatiles are removed under reduced pressure, and the residue is redissolved in 100 mL of EtOAc. The solution is washed successively with 30 mL each of saturated aqueous NaHCO₃, water, and brine. The organic phase is dried over MgSO₄, filtered, and concentrated. Flash chromatography yields the cyclic carbamate product..

25

Example 17

Preparation of Compound of Formula (I): X=H, R₆ = O-3-(quinolin-3-yl)prop-2-ynyl
(Scheme 3)

30

Preparation A: formula (I): X = H, R₂ is -CH₂-CC-(3-quinolyl)

Step 1: 2'-O-Benzoyl-6-O-propargyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate (40 mg), tris(dibenzylideneacetone) dipalladium(0)-chloroform adduct (14 mg), tri-*o*-tolylphosphine (17 mg), copper iodide, and 3-bromoquinoline (72 µl, 0.53 mmol, 10
35 eq) are placed in a round-bottom flask which is flushed with N₂. Degassed acetonitrile (1 mL) and freshly distilled Et₃N (0.015 mL, 0.11 mmol, 2.0 eq) are added. The reaction is refluxed for 63 h. The mixture is returned to ambient temperature and

5 diluted with 40 mL of EtOAc. The solution is washed successively with 10 mL each of saturated aqueous NaHCO_3 , water, and brine. The organic phase is dried over MgSO_4 , filtered, and concentrated. Flash chromatography yields the desired product.

Step 2: The above product is dissolved in 1 mL of methanol, sealed, and
10 refluxed at 80 °C for 16 h. Volatiles are removed under reduced pressure. Flash chromatography yields the desired product.

Preparation B: formula (I): $X = \text{H}$, R_2 is $-\text{CH}_2-\text{CC}-(3-(6\text{-fluoroquinolyl})$

This is prepared according to the method of Preparation A, using 3-bromo-6-
15 fluoroquinoline in place of 3-bromoquinoline.

Preparation C: formula (I): $X = \text{H}$, R_2 is $-\text{CH}_2-\text{CC}-(3-(6\text{-chloroquinolyl})$

This is prepared according to the method of Preparation A, using 3-bromo-6-
chloroquinoline in place of 3-bromoquinoline.

20 Preparation D: formula (I): $X = \text{H}$, R_2 is $-\text{CH}_2-\text{CC}-(4\text{-isoquinolyl})$

This is prepared according to the method of Preparation A, using 4-
bromoisquinoline in place of 3-bromoquinoline.

25 Preparation E: formula (I): $X = \text{H}$, R_2 is $-\text{CH}_2-\text{CC}-(3\text{-pyridyl})$

This is prepared according to the method of Preparation A, using 3-pyridine in
place of 3-bromoquinoline.

Preparation F: formula (I): $X = \text{H}$, R_2 is $-\text{CH}_2-\text{CC}-(3-(6\text{-methylquinolyl})$

30 This is prepared according to the method of Preparation A, using 3-bromo-6-
methylquinoline in place of 3-bromoquinoline.

Preparation G: formula (I): $X = \text{H}$, R_2 is $-\text{CH}_2-\text{CC}-(3-(6\text{-aminoquinolyl})$

This is prepared according to the method of Preparation A, using 3-bromo-6-
35 aminoquinoline in place of 3-bromoquinoline.

Preparation H: formula (I): $X = \text{H}$, R_2 is $-\text{CH}_2-\text{CC}-(3-(5\text{-isoxazol-3-yl})\text{thienyl})$

- 5 This is prepared according to the method of Preparation A, using 5-(isoxazol-3-yl)-2-bromothiophene in place of 3-bromoquinoline.

Preparation I: formula (I): X = H, R₂ is -CH₂-CC-(6-quinolyl)

- 10 This is prepared according to the method of Preparation A, using 6-bromoquinoline in place of 3-bromoquinoline.

Preparation J: formula (I): X = H, R₂ is -CH₂-CC-(3-quinoxal-6-yl)

- 15 This is prepared according to the method of Preparation A using 6-bromoquinoxaline in place of 3-bromoquinoline.

Preparation K: formula (I): X = H, R₂ is -CH₂-CC-(5-(N-(2-pyridyl)-2-furamidyl)

This is prepared according to the method of Preparation A, using N-(2-pyridyl) 5-bromo-2-furamide in place of 3-bromoquinoline.

- 20 Preparation AA: formula (I): X = F, R₂ is -CH₂-CC-(3-quinolyl)

- This was prepared according to the method of Preparation A using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.
- 25

Preparation BB: formula (I): X = F, R₂ is -CH₂-CC-(3-(6-fluoroquinolyl)

- This is prepared according to the method of Preparation B using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.
- 30

Preparation CC: formula (I): X = F, R₂ is -CH₂-CC-(3-(6-chloroquinolyl)

- This is prepared according to the method of Preparation C using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.
- 35

5

Preparation DD: formula (I): X = F, R₂ is -CH₂-CC-(4-isoquinolyl)

This is prepared according to the method of Preparation D using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

Preparation EE: formula (I): X = F, R₂ is -CH₂-CC-(3-pyridyl)

This is prepared according to the method of Preparation E using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

Preparation FF: formula (I): X = F, R₂ is -CH₂-CC-(3-(6-methylquinolyl))

This is prepared according to the method of Preparation F using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

Preparation GG: formula (I): X = F, R₂ is -CH₂-CC-(3-(6-aminoquinolyl))

This is prepared according to the method of Preparation G using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

Preparation HH: formula (I): X = F, R₂ is -CH₂-CC-(3-(5-isoxazol-3-yl)thienyl)

This is prepared according to the method of Preparation H using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

5

Preparation II: formula (I): X = F, R₂ is -CH₂-CC-(6-quinolyl)

This is prepared according to the method of Preparation I using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

Preparation JJ: formula (I): X = F, R₂ is -CH₂-CC-(3-quinoxal-6-yl)

This is prepared according to the method of Preparation J using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

Preparation KK: formula (I): X = F, R₂ is -CH₂-CC-(5-(N-(2-pyridyl)-2-furamidyl)

This is prepared according to the method of Preparation K using 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate in place of 2'-O-benzoyl-6-O-allyl-11-amino-3-descladinosyl-11-deoxy-3-oxo-15-methylerythromycin A 11,12-cyclic carbamate.

25 Example 18

Synthesis of 5-O-(2'-acetyl-desosaminyl)-10,11-anhydro-3,6-dideoxy-3-oxo-14-norerythronolide A

Preparation A: 5-O-desosaminyl-10,11-anhydro-6-deoxy-14-norerythronolide A

A mixture of 6-deoxy-14-norerythromycins A, B, C, and D derived from fermentation (0.5 g) is dissolved in dichloromethane (6 mL) and treated with chlorotrimethylsilane (0.144 mL) and 1-trimethylsilylimidazole (0.20 mL). After 10 minutes, the reaction is treated with 1 N NaOH and is extracted three times with dichloromethane. The organic extracts are combined, washed with sat. NaCl, dried over MgSO₄, filtered, and evaporated to yield a foamy material. This material is dissolved in tetrahydrofuran (5 mL) and treated with 1,1'-carbonyldiimidazole (0.45 g) and sodium hydride (50 mg of a 60% dispersion in oil, washed with hexanes). The mixture is heated at 70°C for 1 hour, then cooled and treated with 1 N NaOH and

5 extracted three times with ethyl acetate. The organic extracts are combined, washed with sat. NaCl, dried over MgSO₄, filtered, and evaporated to dryness. The resulting product mixture is dissolved in ethanol (0.5 mL) and treated with 2% HCl in water (1 mL) to cleave the 3-O-glycosyl groups. The product is recovered by chromatography. Mass spectrometry reveals $[M+H]^+ = 559$.

10

Preparation B: 5-O-(2'-acetyldesosaminy)-10,11-anhydro-6-deoxy-14-norerythronolide A

A solution of 5-O-desosaminy-10,11-anhydro-6-deoxy-14-norerythronolide A (0.5 g) in acetone (10 mL) is treated with acetic anhydride (0.10 mL) and potassium carbonate (0.15 g) at ambient temperature for 24 hours, filtered, and concentrated to dryness to yield the product. Mass spectrometry reveals $[M+H]^+ = 601$.

Preparation C: 5-O-(2'-acetyldesosaminy)-10,11-anhydro-3,6-dideoxy-3-oxo-14-norerythronolide A

20 A solution of 5-O-(2'-acetyldesosaminy)-10,11-anhydro-6-deoxy-14-norerythronolide A (0.5 g) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.0 g) in dichloromethane (10 mL) is treated with methylsulfoxide (1.0 mL) and cooled to 5 °C. A solution of pyridinium trifluoroacetate (1.0 g) in dichloromethane (10 mL) is added dropwise, and the mixture is stirred at 5 °C for 25 hours. The mixture is diluted with ethyl acetate, washed with water and saturated NaCl then dried over MgSO₄, filtered, and evaporated to dryness. The product is purified by chromatography. Mass spectrometry reveals $[M+H]^+ = 599$.

Example 19

30 Synthesis of 5-O-(2'-acetyldesosaminy)-10,11-anhydro-3,6-dideoxy-3-oxo-14,15-dehydrorerythronolide A

Preparation A: 5-O-desosaminy-10,11-anhydro-6-deoxy-14,15-dehydroerythronolide A

35 A mixture of 6-deoxy-14,15-dehydroerythromycins A, B, C, and D derived from fermentation (0.5 g) is dissolved in dichloromethane (6 mL) and treated with chlorotrimethylsilane (0.144 mL) and 1-trimethylsilylimidazole (0.20 mL). After 10

5 minutes, the reaction is treated with 1 N NaOH and is extracted three times with dichloromethane. The organic extracts are combined, washed with sat. NaCl, dried over MgSO₄, filtered, and evaporated to yield a foamy material. This material is dissolved in tetrahydrofuran (5 mL) and treated with 1,1'-carbonyldiimidazole (0.45 g) and sodium hydride (50 mg of a 60% dispersion in oil, washed with hexanes). The mixture is heated at 70°C for 1 hour, then cooled and treated with 1 N NaOH and extracted three times with ethyl acetate. The organic extracts are combined, washed with sat. NaCl, dried over MgSO₄, filtered, and evaporated to dryness. The resulting product mixture is dissolved in ethanol (0.5 mL) and treated with 2% HCl in water (1 mL) to cleave the 3-O-glycosyl groups. The product is recovered by chromatography.

15 Preparation B: 5-O-(2'-acetyl-desosaminyl)-10,11-anhydro-6-deoxy-14,15-dehydroerythronolide A

A solution of 5-O-desosaminyl-10,11-anhydro-6-deoxy-14,15-dehydroerythronolide A (0.5 g) in acetone (10 mL) is treated with acetic anhydride (0.10 mL) and potassium carbonate (0.15 g) at ambient temperature for 24 hours, filtered, and concentrated to dryness to yield the product.

Preparation C: 5-O-(2'-acetyl-desosaminyl)-10,11-anhydro-3,6-dideoxy-3-oxo-14,15-dehydroerythronolide A

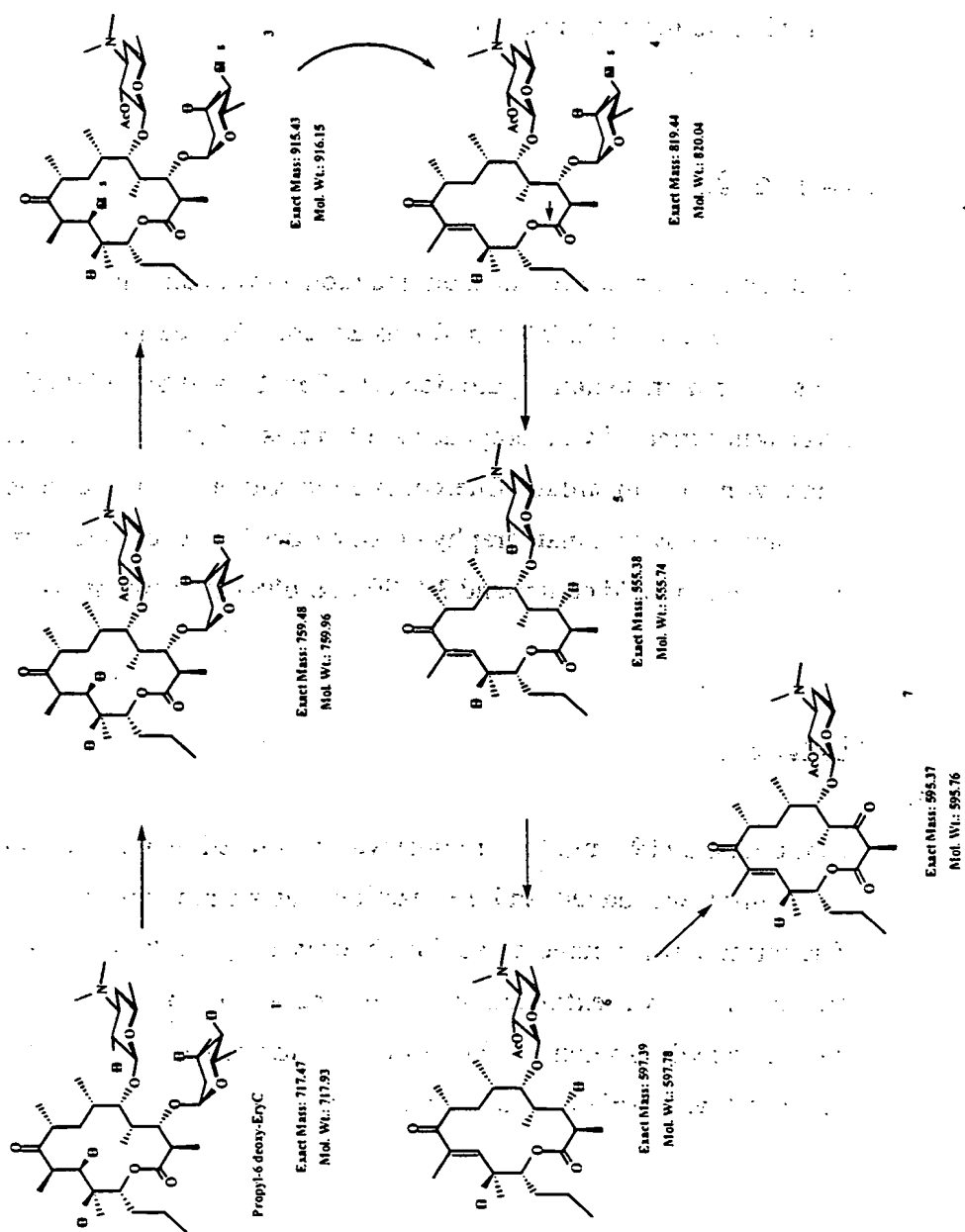
25 A solution of 5-O-(2'-acetyl-desosaminyl)-10,11-anhydro-6-deoxy-14,15-dehydroerythronolide A (0.5 g) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.0 g) in dichloromethane (10 mL) is treated with methylsulfoxide (1.0 mL) and cooled to 5 °C. A solution of pyridinium trifluoroacetate (1.0 g) in dichloromethane (10 mL) is added dropwise, and the mixture is stirred at 5 °C for 2 hours. The mixture is diluted with ethyl acetate, washed with water and saturated NaCl then dried over MgSO₄, filtered, and evaporated to dryness. The product is purified by chromatography.

5

Example 20Synthesis of 5-O-(2'-acetylidesosaminyl)-10,11-anhydro-3,6-dideoxy-3-oxo-15-methylerythronolide A

The title compound was prepared in accordance with the following scheme:

Scheme 6



5

Reaction 1

To a solution of compound 1 (220mg, 0.307mmol) in dichloromethane (5mL) were
10 added potassium carbonate (50mg) and acetic anhydride (100_ L, 0.9mmol), and the
reaction was stirred at room temperature for 16 hours. The solution was filtered,
sodium hydroxide (1N, 25mL) and brine (25mL) added and the aqueous layer was
extracted with ethyl acetate 6 times. The combined organic layers were dried with
sodium sulfate, filtered, and the solvent removed *in vacuo*. The crude product 2 was
15 carried on to the next step.

Reaction 2:

20 Compound 2 (crude product from reaction 1) was dissolved in pyridine (5mL) and
mesyl chloride (70_ L, 0.9mmol) was added. The reaction was stirred at -20°C for 2
days, poured on sodium hydroxide (1N, 25mL) and brine (25mL) and the aqueous
layer was extracted with ethyl acetate 6 times. The combined organic layers were
dried with sodium sulfate, filtered, and the solvent removed *in vacuo*. The residue
25 was purified by chromatography on silica gel (toluene/acetone = 3:1, 1% ammonium
hydroxide) to yield compound 3 (190 mg, 68% over two steps).

Reaction 3:

30

Compound 3 (190 mg, 0.21 mmol) was dissolved in acetone (7mL) and DBU (63_ L,
0.42 mmol) was added, and the reaction was stirred at room temperature over night.
The mixture was poured on sodium hydroxide (1N, 25mL) and brine (25mL) and the
aqueous layer was extracted with ethyl acetate 6 times. The combined organic layers
35 were dried with sodium sulfate, filtered, and the solvent removed *in vacuo*. The crude
product 4 was carried on to the next step.

5 Reaction 4:

To Compound 4 (crude product from step before) was added hydrochloric acid (30 mL, 3N) and ethanol (2mL) and the mixture was stirred vigorously for 6 hours.

10 Sodium hydroxide (5mL, 10N) was added and the aqueous layer was extracted with ethyl acetate 6 times. The combined organic layers were dried with sodium sulfate, filtered, and the solvent removed *in vacuo*. The crude product 5 was carried on to the next step.

15 Reaction 5:

To Compound 5 (crude product from step before) in dichloromethane (5mL) was added acetic anhydride (50 μ L, 0.45mmol) and potassium carbonate (100mg) and the mixture was stirred vigorously for 9 hours. The reaction was filtered, sodium

20 hydroxide (20mL, 1N) and brine (25mL) were added and the aqueous layer was extracted with ethyl acetate 6 times. The combined organic layers were dried with sodium sulfate, filtered, and the solvent removed *in vacuo*. The residue was purified by chromatography on silica gel (toluene/acetone = 3:1, 1% ammonium hydroxide) to yield compound 6 (110 mg, 89% over three steps).

25

Reaction 6:

Compound 6 (110mg, 0.184 mmol) was dissolved in dichloromethane (10mL) and Dess-Martin reagent (220 mg, 0.53 mmol) was added. The reaction was stirred at
30 room temperature for 45 min. The reaction was quenched with Sodium hydroxide (20mL, 1N) and brine (25mL) and the aqueous layer was extracted with ethyl acetate 6 times. The combined organic layers were dried with sodium sulfate, filtered, and the solvent removed *in vacuo*. The residue was purified by chromatography on silica gel
35 (toluene/acetone, gradient = 6:1 - 3:1, 1% ammonium hydroxide) to yield compound 7 (94 mg, 86%)

5

Example 21Synthesis of 1-(4-amino-2-butenyl)-1H-imidazo[4,5-b]pyridinePreparation A: (E)-N-(4-bromo-2-butenyl)phthalimide

10 A solution of 1,4-dibromo-2-butene (23g, 107.9 mmol) and potassium carbonate (16.39 g, 118.7 mmol) in DMF (50 mL) at room temperature was treated with potassium phthalimide (10g, 53.9 mmol). After 10 min., the reaction mixture was allowed to stir for 24h, filtered, and concentrated in vacuo. The resulting oil was diluted with ethyl acetate (200 mL), washed with saturated aqueous sodium
15 monobasic phosphate (2 x 100 mL), dried (MgSO₄), and concentrated in vacuo to afford a reddish oil. Purification by flash chromatography (0-20% ethyl acetate/hexanes) afforded 5.73g of the title compound; mass (CI) m/z = 303 (M+H).

Preparation B: 1-[(E)-4-phthalimido-2-butenyl]-1H-imidazo[4,5-b]pyridine and 3-[(E)-4-phthalimido-2-butenyl]-3H-imidazo[4,5-b]pyridine

20 A slurry of NaH (1.02g, 25.4 mmol) in DMF (50 mL) at room temperature was treated with 4-azabenzimidazole (2.90g, 24.4 mmol). After 10 min., the reaction mixture was treated with a solution of (E)-N-(4-bromo-2-butenyl)phthalimide (5.7g, 20.3 mmol) in DMF (5 mL) over 30 min. The reaction mixture was allowed to stir for
25 1h, then quenched by careful addition of water (5 mL) and the reaction mixture was concentrated in vacuo. The resulting residue was diluted with CH₂Cl₂ (50 mL), washed with brine (2 x 25 mL), dried (MgSO₄), and concentrated in vacuo to afford an off-white solid. Purification by flash chromatography (ethyl acetate containing 3% NH₄OH) afforded 2.08g of 3-[(E)-4-phthalimido-2-butenyl]-3H-imidazo[4,5-
30 b]pyridine. Changing the chromatography solvent to 5% methanol/ethyl acetate containing 5% NH₄OH afforded 1.66g of 1-[(E)-4-phthalimido-2-butenyl]-1H-imidazo[4,5-b]pyridine; mass (CI) m/z = 319 (M+H).

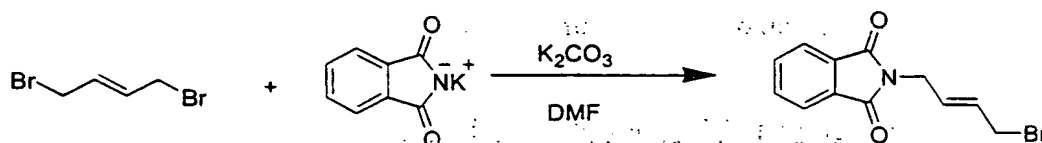
Preparation C: 1-(4-amino-2-butenyl)-1H-imidazo[4,5-b]pyridine

35 A solution of 1-[(E)-4-phthalimido-2-butenyl]-1H-imidazo[4,5-b]pyridine (2.19g, 6.87 mmol) in ethanol (100 mL) at room temperature was treated with hydrazine monohydrate (3.33 mL, 68.7 mmol). After 10 min., the reaction mixture

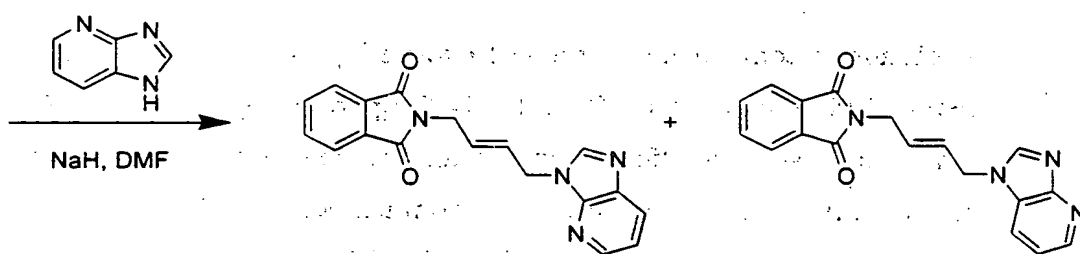
- 5 was warmed to 60°C for 2h, allowed to cool to 25°C, and recooled to 0°C in an ice bath. The resulting slurry was filtered, and the filtrate was concentrated in vacuo. Purification by flash chromatography (6% NH₄OH/ethanol) afforded 0.92g of the title compound; mass (CI) m/z = 211 (M+H).

Scheme 7

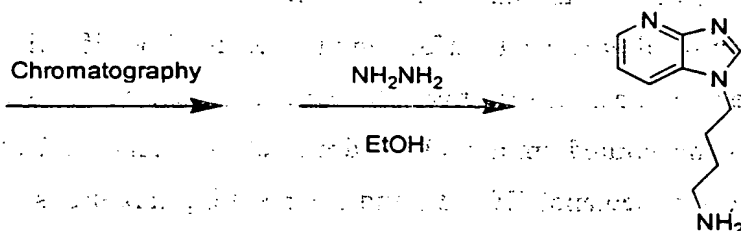
10



15



20



25

30

Example 22

Compound I where R₆ = OCH₃, R₁₃ = n-propyl, X = H, R = 1H-imidazo[4,5-b]pyridin-1-ylbutyl (Compound K in Table 1)

35

Step 1: Compound 19 (Scheme 2) where R₂ = CH₃, R₁₃ = n-propyl, X = H, R₂ = acetyl

A solution of compound of formula 18 (Scheme 2) where R_a = CH₃, R₁₃ = n-propyl, X = H, R₂ = acetyl (625 mg, 1.00 mmol) in N,N-dimethylformamide (8 mL) at -10 °C

5 under nitrogen is treated with sodium hydride (60 wt % in mineral oil, 80 mg, 2.00 mmol). After 30 minutes, the resulting reaction mixture is treated with 1,1'-carbonyldiimidazole (490 mg, 3.02 mmol) and the reaction mixture is allowed to stir for 2 hours at -10 °C. The reaction mixture is quenched with water (30 mL) and extracted with ether (3 x 30 mL). The combined organic layers are washed with water
10 (30 mL) and brine (30 mL), dried with magnesium sulfate, and concentrated *in vacuo* to afford 3 as an off-white foam.

Step 2: Compound I where $R_6 = \text{OCH}_3$, $R_{13} = \text{n-propyl}$, $X = \text{H}$, $R = 1H\text{-imidazo}[4,5\text{-}b]\text{pyridin-1-ylbutyl}$

15

A solution of compound of formula 19 where $R_a = \text{CH}_3$, $R_{13} = \text{n-propyl}$, $X = \text{H}$, $R_2 = \text{acetyl}$ (1.00 mmol) and 1*H*-imidazo[4,5-*b*]pyridin-1-ylbutylamine (570 mg, 3.00 mmol) in *N,N*-dimethylformamide (4 mL) is heated to 60 °C for 24 hours. The reaction mixture is allowed to cool to room temperature, diluted with water (30 mL),
20 and extracted with ethyl acetate (3 x 30 mL). The combined organic layers are washed with water (2 x 30 mL) and brine (30 mL), dried with magnesium sulfate, filtered, and concentrated *in vacuo* to afford an oily residue. (Purification of 2'-acetyl I can be achieved by flash chromatography (0-5% methanol in dichloromethane containing 1-2% concentrated ammonium hydroxide) on a silica gel column.) The
25 residue is dissolved in methanol (20 mL) and the resulting mixture is allowed to stir for 18 hours at room temperature. The reaction mixture is concentrated *in vacuo* and purification of I is achieved by flash chromatography on silica gel (95:5:0.5 dichloromethane/methanol/concentrated ammonium hydroxide) to provide the title compound (487 mg, 61%).

30

Example 23

Compound I where $R_6 = \text{OCH}_3$, $R_{13} = \text{n-propyl}$, $X = \text{F}$, $R = 1H\text{-imidazo}[4,5\text{-}b]\text{pyridin-1-ylbutyl}$ (Compound O in Table 1)

35

The title compound was prepared as described in Example 21 except that the compound of formula 18 (Scheme 2) where $R_a = \text{CH}_3$, $R_{13} = \text{n-propyl}$, $X = \text{F}$, $R_2 =$

- 5 acetyl was substituted for the compound of formula 18 (Scheme 2) where $R_4 = \text{CH}_3$, $R_{13} = n\text{-propyl}$, $X = \text{H}$, $R_2 = \text{acetyl}$. Yield = 25%.

The following compounds can also be prepared by analogous procedures and methods as described in Example 22.

10

Compound of formula I where $X = \text{H}$, $R_6 = \text{H}$, $R_{13} = \text{methyl}$, $R = \text{quinolin-4-ylbutyl}$

Compound of formula I where $X = \text{F}$, $R_6 = \text{H}$, $R_{13} = \text{methyl}$, $R = \text{quinolin-4-ylbutyl}$

Compound of formula I where $X = \text{H}$, $R_6 = \text{OCH}_3$, $R_{13} = \text{methyl}$, $R = \text{quinolin-4-ylbutyl}$ (A)

- 15 Compound of formula I where $X = \text{F}$, $R_6 = \text{OCH}_3$, $R_{13} = \text{methyl}$, $R = \text{quinolin-4-ylbutyl}$

Compound of formula I where $X = \text{H}$, $R_6 = \text{H}$, $R_{13} = \text{methyl}$, $R = 4\text{-phenylimidazol-1-ylbutyl}$

Compound of formula I where $X = \text{F}$, $R_6 = \text{H}$, $R_{13} = \text{methyl}$, $R = 4\text{-phenylimidazol-1-ylbutyl}$

20

Compound of formula I where $X = \text{H}$, $R_6 = \text{OCH}_3$, $R_{13} = \text{methyl}$, $R = 4\text{-phenylimidazol-1-ylbutyl}$

Compound of formula I where $X = \text{F}$, $R_6 = \text{OCH}_3$, $R_{13} = \text{methyl}$, $R = 4\text{-phenylimidazol-1-ylbutyl}$

- 25 Compound of formula I where $X = \text{H}$, $R_6 = \text{H}$, $R_{13} = \text{methyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$

Compound of formula I where $X = \text{F}$, $R_6 = \text{H}$, $R_{13} = \text{methyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$

Compound of formula I where $X = \text{H}$, $R_6 = \text{OCH}_3$, $R_{13} = \text{methyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$ (B)

30

Compound of formula I where $X = \text{F}$, $R_6 = \text{OCH}_3$, $R_{13} = \text{methyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$

Compound of formula I where $X = \text{H}$, $R_6 = \text{H}$, $R_{13} = \text{methyl}$, $R = \text{pyridin-4-ylbutyl}$

Compound of formula I where $X = \text{F}$, $R_6 = \text{H}$, $R_{13} = \text{methyl}$, $R = \text{pyridin-4-ylbutyl}$

- 35 Compound of formula I where $X = \text{H}$, $R_6 = \text{OCH}_3$, $R_{13} = \text{methyl}$, $R = \text{pyridin-4-ylbutyl}$

Compound of formula I where $X = \text{F}$, $R_6 = \text{OCH}_3$, $R_{13} = \text{methyl}$, $R = \text{pyridin-4-ylbutyl}$

- 5 Compound of formula I where $X = H$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = 3H\text{-imidazo}[4,5\text{-}b]\text{pyridin-3-ylbutyl}$
Compound of formula I where $X = F$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = 3H\text{-imidazo}[4,5\text{-}b]\text{pyridin-3-ylbutyl}$
Compound of formula I where $X = H$, $R_6 = \text{OCH}_3$, $R_{13} = \text{methyl}$, $R = 3H\text{-imidazo}[4,5\text{-}b]\text{pyridin-3-ylbutyl}$ (C)
10 Compound of formula I where $X = F$, $R_6 = \text{OCH}_3$, $R_{13} = \text{methyl}$, $R = 3H\text{-imidazo}[4,5\text{-}b]\text{pyridin-3-ylbutyl}$
Compound of formula I where $X = H$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = 1H\text{-imidazo}[4,5\text{-}b]\text{pyridin-1-ylbutyl}$
15 Compound of formula I where $X = F$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = 1H\text{-imidazo}[4,5\text{-}b]\text{pyridin-1-ylbutyl}$
Compound of formula I where $X = H$, $R_6 = \text{OCH}_3$, $R_{13} = \text{methyl}$, $R = 1H\text{-imidazo}[4,5\text{-}b]\text{pyridin-1-ylbutyl}$ (D)
Compound of formula I where $X = F$, $R_6 = \text{OCH}_3$, $R_{13} = \text{methyl}$, $R = 1H\text{-imidazo}[4,5\text{-}b]\text{pyridin-1-ylbutyl}$
20 Compound of formula I where $X = H$, $R_6 = H$, $R_{13} = n\text{-propyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$ (P)
Compound of formula I where $X = F$, $R_6 = H$, $R_{13} = n\text{-propyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$
25 Compound of formula I where $X = H$, $R_6 = \text{OCH}_3$, $R_{13} = n\text{-propyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$ (H)
Compound of formula I where $X = F$, $R_6 = \text{OCH}_3$, $R_{13} = n\text{-propyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$
30 Compound of formula I where $X = H$, $R_6 = \text{OCH}_3$, $R_{13} = n\text{-propyl}$, $R = 1H\text{-imidazo}[4,5\text{-}c]\text{pyridin-1-ylbutyl}$ (M)
Compound of formula I where $X = F$, $R_6 = \text{OCH}_3$, $R_{13} = n\text{-propyl}$, $R = 1H\text{-imidazo}[4,5\text{-}c]\text{pyridin-1-ylbutyl}$
Compound of formula I where $X = H$, $R_6 = \text{OCH}_3$, $R_{13} = n\text{-propyl}$, $R = 3H\text{-imidazo}[4,5\text{-}c]\text{pyridin-3-ylbutyl}$
35 Compound of formula I where $X = F$, $R_6 = \text{OCH}_3$, $R_{13} = n\text{-propyl}$, $R = 3H\text{-imidazo}[4,5\text{-}c]\text{pyridin-3-ylbutyl}$

- 5 Compound of formula I where $X = H$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = \text{purin-7-ylbutyl}$
Compound of formula I where $X = F$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = \text{purin-7-ylbutyl}$
Compound of formula I where $X = H$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = \text{purin-9-ylbutyl}$
(N)
Compound of formula I where $X = F$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = \text{purin-9-ylbutyl}$
- 10 Compound of formula I where $X = H$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 1H$ -imidazo[4,5-b]pyridin-1-ylbut-2-enyl (L)
Compound of formula I where $X = F$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 1H$ -imidazo[4,5-b]pyridin-1-ylbut-2-enyl
Compound of formula I where $X = H$, $R_6 = H$, $R_{13} = n$ -propyl, $R = 4$ -(pyrimidin-5-yl)imidazol-1-ylbutyl
- 15 Compound of formula I where $X = F$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 4$ -(pyrimidin-5-yl)imidazol-1-ylbutyl
Compound of formula I where $X = H$, $R_6 = H$, $R_{13} = n$ -propyl, $R = 1H$ -imidazo[4,5-b]pyridin-1-ylbutyl (Q)
- 20 Compound of formula I where $X = F$, $R_6 = H$, $R_{13} = n$ -propyl, $R = 1H$ -imidazo[4,5-b]pyridin-1-ylbutyl
Compound of formula I where $X = H$, $R_6 = H$, $R_{13} = n$ -propyl, $R = 3H$ -imidazo[4,5-b]pyridin-3-ylbutyl
Compound of formula I where $X = F$, $R_6 = H$, $R_{13} = n$ -propyl, $R = 3H$ -imidazo[4,5-b]pyridin-3-ylbutyl
- 25 Compound of formula I where $X = H$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 3H$ -imidazo[4,5-b]pyridin-3-ylbutyl (J)
Compound of formula I where $X = F$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 3H$ -imidazo[4,5-b]pyridin-3-ylbutyl
- 30 Compound of formula I where $X = H$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 4$ -phenylimidazol-1-ylbutyl
Compound of formula I where $X = F$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 4$ -phenylimidazol-1-ylbutyl
Compound of formula I where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 4$ -phenylimidazol-1-ylbutyl
- 35 Compound of formula I where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 4$ -phenylimidazol-1-ylbutyl

- 5 Compound of formula I where $X = H$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$
Compound of formula I where $X = F$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$
Compound of formula I where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$ (E)
10 Compound of formula I where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$
Compound of formula I where $X = H$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyrimidin-5-yl)imidazol-1-ylbutyl}$
15 Compound of formula I where $X = F$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyrimidin-5-yl)imidazol-1-ylbutyl}$
Compound of formula I where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyrimidin-5-yl)imidazol-1-ylbutyl}$
Compound of formula I where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyrimidin-5-yl)imidazol-1-ylbutyl}$
20 Compound of formula I where $X = H$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 3H\text{-imidazo[4,5-b]pyridin-3-ylbutyl}$
Compound of formula I where $X = F$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 3H\text{-imidazo[4,5-b]pyridin-3-ylbutyl}$
25 Compound of formula I where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 3H\text{-imidazo[4,5-b]pyridin-3-ylbutyl}$ (F)
Compound of formula I where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 3H\text{-imidazo[4,5-b]pyridin-3-ylbutyl}$
Compound of formula I where $X = H$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = 1H\text{-imidazo[4,5-b]pyridin-1-ylbutyl}$
30 Compound of formula I where $X = F$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = 1H\text{-imidazo[4,5-b]pyridin-1-ylbutyl}$
Compound of formula I where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{methyl}$, $R = 1H\text{-imidazo[4,5-b]pyridin-1-ylbutyl}$ (G)
35 Compound of formula I where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{methyl}$, $R = 1H\text{-imidazo[4,5-b]pyridin-1-ylbutyl}$

Example 24Synthesis of 2'-O-benzoyl-6-O-allyl-3-descladinosyl-3-oxo-11-deoxy-11-amino-2-fluoro-15-methylerythromycin A 11,12-cyclic carbamate

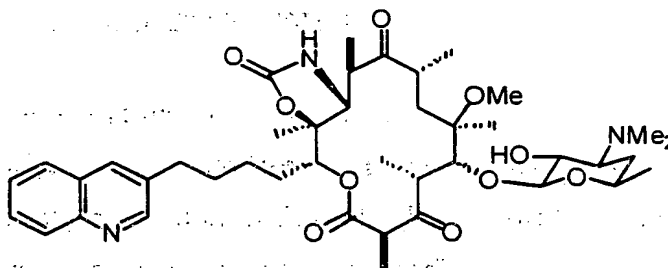
To a THF solution (0.5 ml) of 2'-O-benzoyl-6-O-allyl-3-descladinosyl-3-oxo-11-deoxy-11-amino-15-methylerythromycin A 11,12-cyclic carbamate (100 mg, 0.132 mmol, 1.0 eq) was added a THF solution of potassium *tert*-butoxide (0.3 ml, 1M, 2.3 eq.) at -78°C. The reaction mixture was then kept at -60°C to -40°C for 20 min., followed by introduction of N-Fluorobenzenesulfonimide (46 mg, 0.146 mmol, 1.1 eq.) in THF (0.2 ml) at -78°C. The reaction mixture was kept at -70°C to -40°C for 1 h before it was allowed to warm to 0°C from -70°C in 1.5 h. It was then diluted with EtOAc, washed with saturated aqueous NaHCO₃, water, and brine. The organic phase was dried over MgSO₄, filtered, and concentrated. Flash chromatography of the crude product (4:1 hexanes:acetone + 1% Et₃N) yielded 76 mg (74%) of the desired product. ¹³C-NMR (100.6 MHz, CDCl₃) δ 217.5, 203 (d, *J* = 27.6 Hz), 165.5 (d, *J* = 23.8 Hz), 165.2, 157.5, 135.4, 132.9, 130.4, 129.8, 128.3, 118.0, 101.7, 98 (d, *J* = 207 Hz), 83.5, 79.1, 78.6, 72.1, 69.4, 64.6, 63.5, 57.5, 44.2, 40.7, 40.4, 38.5, 37.3, 31.4, 31.3, 24.9 (d, *J* = 24.3 Hz), 21.0, 20.7, 19.4, 17.7, 15.0, 13.9, 13.7, 13.3.

Example 25Synthesis of 2'-O-benzoyl-6-O-propargyl-3-descladinosyl-3-oxo-10,11-anhydro-2-fluoro-15-methylerythromycin A

A solution of 2'-O-benzoyl-6-O-propargyl-3-descladinosyl-3-oxo-10,11-anhydro-15-methyl-erythromycin A tetrahydrofuran under inert atmosphere is cooled to -78 °C and treated with 1.0 M potassium *tert*-butoxide in tetrahydrofuran. The mixture is stirred for 5 minutes, and a solution of N-fluorobenzenesulfonimide in tetrahydrofuran is added in three portions over 2 hours. After addition, the reaction is allowed to warm to ambient temperature and kept for an additional 5 hours. Aqueous K₂CO₃ is added, and the mixture is extracted with CH₂Cl₂. The organic extracts are combined, dried over MgSO₄, filtered, and evaporated. Chromatography on silica gel gives the product.

Example 26

15-(2-(3-quinolyl)ethyl)-3-descladinosyl-3-oxo-6-O-methylerythromycin A
11,12-cyclic carbamate



(A) 15-(2-(3-quinolyl)ethyl)erythromycin A-9-oxime

15-(2-(3-quinolyl)ethyl)erythromycin A (25.7 g, 28.9 mmol, 1.00 eq) is suspended in 42 mL of 2-propanol. Hydroxylamine (50 wt% in H₂O, 22.2 mL, 375 mmol, 13.0 eq) is added. The mixture is stirred until homogeneous. Glacial HOAc is added. The solution is stirred at 50 °C for 11 h. Saturated NaHCO₃ is added. The mixture is concentrated and extracted with CHCl₃ (4x400 mL); washed with NaHCO₃ and water. The combined aqueous layers are back-extracted with 400 mL CHCl₃. The combined organic phases are washed with brine, dried over Na₂SO₄, filtered, and concentrated to yield the crude material. This is carried on without further purification.

(B) 15-(2-(3-quinolyl)ethyl)erythromycin A-9-(isopropoxycyclohexyl)oxime

The crude 15-(2-(3-quinolyl)ethyl)erythromycin A-9-oxime from above is dissolved in 72 mL of anhydrous CH₂Cl₂, and 1,1-diisopropoxycyclohexane (29.2 mL, 140 mmol, 4.86 eq) is added dropwise. A solution of pyridinium p-toluenesulfonate (10.5 g, 41.9 mmol, 1.45 eq) in CH₂Cl₂ (36 mL) is added dropwise. Dichloromethane (200 mL) is added after 15 h. The solution is washed with NaHCO₃ (2x100 mL) and water (100 mL). The combined aqueous phases are back-extracted with 100 mL CH₂Cl₂. The combined organic layers are washed with brine, dried over MgSO₄, filtered, and concentrated. The material is chromatographed over silica gel to give the desired product.

(C) 2',4''-Bis(O-trimethylsilyl)- 15-(2-(3-quinolyl)ethyl)erythromycin A-9-(isopropoxycyclohexyl)oxime.

5 The 15-(2-(3-quinolyl)ethyl)erythromycin A-9-(isopropoxycyclohexyl)oxime (22.2 g, 21.3 mmol, 1.0 eq) is dissolved in 54 mL anhydrous CH_2Cl_2 and cooled in an ice/water bath. A mixture of chlorotrimethylsilane (4.05 mL, 31.9 mmol, 1.5 eq), N-(trimethylsilyl)-imidazole (7.81 mL, 53.2 mmol, 2.5 eq), and CH_2Cl_2 (18 mL) is added dropwise. The reaction is stirred for 15 minutes after complete addition and quenched
10 with 600 mL EtOAc. The mixture is washed with sat. NaHCO_3 (2x200 mL), water (200 mL), and brine (200 mL). The organic layer is dried over MgSO_4 , filtered, and concentrated to yield the crude product which was carried on without further purification.

15 (D) 2',4''-Bis(O-trimethylsilyl)-6-O-methyl-15-(2-(3-quinolyl)ethyl)erythromycin A-9-(isopropoxycyclohexyl)oxime

Crude 2',4''-bis(O-trimethylsilyl)-15-(2-(3-quinolyl)ethyl)erythromycin A-9-(isopropoxycyclohexyl)oxime is dissolved in anhydrous tetrahydrofuran (41 mL) and cooled to 10 °C. Anhydrous methylsulfoxide (41.4 mL) and methyl bromide (2.0 M
20 in ether, 20.7 mL, 41.4 mmol, 2.0 eq) are added. A 1.0 M solution of potassium t-butoxide in THF (41.4 mL, 41.4 mmol, 2.0 eq) is diluted with anhydrous methylsulfoxide (41.4 mL). This is added to the reaction mixture at a rate of 0.5 eq/hr. The reaction is monitored by TLC (5:1 toluene:acetone). The reaction is quenched by the addition of ethyl acetate (200 mL) and sat. NaHCO_3 (70 mL). The
25 mixture is transferred to a separatory funnel and diluted with 850 mL of ethyl acetate. The organic phase is washed with sat. NaHCO_3 , water, and brine (300mL each). The resulting emulsion is filtered through Celite. The separated organic phase is then dried over MgSO_4 , filtered, and concentrated to give the crude product which is carried on without further purification.

30

(E) 6-O-Methyl-15-(2-(3-quinolyl)ethyl)erythromycin A-9-oxime

The crude 2',4''-bis(trimethylsilyl)-6-O-methyl-15-(2-(3-quinolyl)ethyl)erythromycin A-9-(isopropoxycyclohexyl)oxime from above is dissolved in acetonitrile (110 mL). Glacial acetic acid (67 mL) diluted with water (55 mL) is added slowly. The solution
35 is stirred 8 h. Toluene and 2-propanol are added, and the solution is concentrated. The product is then dissolved in toluene and concentrated twice to give the crude product which was carried on without further purification.

5

(F) 6-O-methyl-15-(2-(3-quinolyl)ethyl)erythromycin A

The crude 6-O-methyl-15-(2-(3-quinolyl)ethyl)erythromycin A-9-oxime from above and sodium hydrosulfite (23.1 g, 113 mmol, 5.63 eq) are placed in a round-bottom flask equipped with a condenser and flushed with N₂. Ethanol (140 mL) and water
10 (140 mL) are added. Formic acid (3.75 mL, 95.4 mmol, 4.77 eq) is added dropwise. The mixture is stirred at 80 C for 4.5 h. After the solution returned to room temperature, sat. NaHCO₃ was added. The pH is adjusted to 9-10 with 6 N NaOH. The mixture is then extracted with 3x400 mL of ethyl acetate. The combined organic phases are washed with sat. NaHCO₃ then water (250 mL each). The combined
15 aqueous phases are back-extracted with ethyl acetate (400 mL). The combined organic phases are washed with brine, dried over MgSO₄, filtered, and concentrated to give the crude product which was carried on without further purification. Pure product can be obtained by chromatography on silica gel.

20 (G) 6-O-Methyl-15-(2-(3-quinolyl)ethyl)-3-descladinosylerythromycin A

The crude 6-O-methyl-15-(2-(3-quinolyl)ethyl)erythromycin A is stirred in 280 mL of 0.5 M HCl for 3 h. The pH is adjusted to 9-10 with 6 N NaOH. The precipitate is collected by vacuum filtration and washed with water. The mother liquor is extracted with 3x400 mL ethyl acetate. The combined organic phases are washed with sat.
25 NaHCO₃ and water. The combined aqueous phases are back-extracted with ethyl acetate. The combined organic phases are washed with brine, dried over MgSO₄, filtered, and concentrated. The combined product is chromatographed over silica gel the desired product as a white solid.

30 (H) 2'-O-Acetyl-6-O-methyl-15-(2-(3-quinolyl)ethyl)-3-descladinosylerythromycin A

6-O-Methyl-15-(2-(3-quinolyl)ethyl)-3-descladinosyl erythromycin A (11.5 g, 15.5 mmol, 1.0 eq) is dissolved in 40 mL ethyl acetate. A solution of acetic anhydride (2.92 mL, 31.0 mmol, 2.0 eq) in ethyl acetate (35 mL) is added dropwise. The reaction is stirred for 30 min and then concentrated. The material is chromatographed
35 over silica gel to give the desired product as a white solid.

5 (I) 2'-O-Acetyl-3-descladinosyl-3-oxo-6-O-methyl-15-(2-(3-quinolyl)ethyl)erythromycin A

2'-O-Acetyl-6-O-methyl-15-(2-(3-quinolyl)ethyl)-3-descladinosyl erythromycin A (10 g, 12.8 mmol, 1.0 eq) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (16.51 g, 86.1 mmol, 6.7 eq) are combined in a round-bottom flask and
10 flushed with N₂. The solids are dissolved in anhydrous CH₂Cl₂ (64 mL) and cooled in an ice water bath. Anhydrous DMSO (15.5 mL, 218 mmol, 17 eq) is added. A solution of pyridinium trifluoroacetate (12.14 g, 62.9 mmol, 4.9 eq) in CH₂Cl₂ (47 mL) is added over 3 h. The solution is diluted with 600 mL of ethyl acetate and washed with sat. NaHCO₃, water, and brine (200 mL each). The organic phase is
15 dried over MgSO₄, filtered, and concentrated. Chromatography over silica gel gives the desired product.

(J) 2'-O-Acetyl-3-oxo-3-descladinosyl-11-methanesulfonyl-6-O-methyl-15-(2-(3-quinolyl)ethyl)erythromycin A

20 2'-O-Acetyl-3-descladinosyl-3-oxo-6-O-methyl-15-(2-(3-quinolyl)ethyl)erythromycin A is dissolved in freshly distilled pyridine (35 mL) and cooled in an ice water bath. Methanesulfonyl chloride is added dropwise. The reaction is allowed to come to ambient temperature and stirred overnight. Ethyl acetate (700 mL) is added, and the solution is washed with sat. NaHCO₃, water, and brine (200 mL each). The organic
25 phase is dried over MgSO₄, filtered, and concentrated. Chromatography over silica gel gives the desired compound.

(K) 2'-O-Acetyl-10,11-anhydro-3-descladinosyl-3-oxo-6-O-methyl-15-(2-(3-quinolyl)ethyl)erythromycin A

30 2'-O-Acetyl-3-oxo-3-descladinosyl-11-methanesulfonyl-6-O-methyl-15-(2-(3-quinolyl)ethyl)erythromycin A (6 g, 6.98 mmol, 1.0 eq) is dissolved in acetone (23 mL). 1,8-Diazabicyclo(5.4.0)undec-7-ene (5.22 mL, 34.9 mmol, 5.0 eq) is added dropwise. The reaction is stirred at ambient temperature for 4 h and then concentrated. Chromatography over silica gel gave the desired compound.

35

(L) 3-descladinosyl-3-oxo-6-O-methyl-15-(2-(3-quinolyl)ethyl)erythromycin A 11,12-cyclic carbamate

5 A solution of 2'-O-Acetyl-10,11-anhydro-3-descladinosyl-3-oxo-6-O-methyl-15-(2-(3-quinolyl)ethyl)erythromycin A in dry tetrahydrofuran is added to a stirred suspension of NaH (3 eq.) in THF cooled to -10 °C. To this is added a solution of carbonyldiimidazole (10 eq.) in THF/DMF (5:3), and the mixture is stirred for 2 hours. The reaction is warmed to ambient temperature and diluted with concentrated aqueous ammonia and stirred overnight. The mixture is diluted with ethyl acetate and washed with aq. NaHCO₃ and brine, dried over MgSO₄, and evaporated. Chromatography on silica gel yields the product.

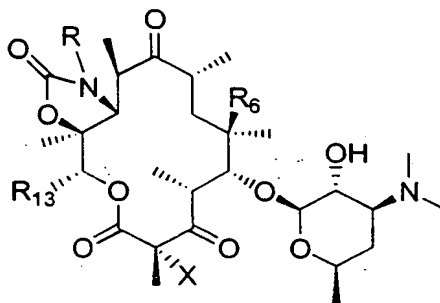
Example 27

In Vitro Susceptibility Testing

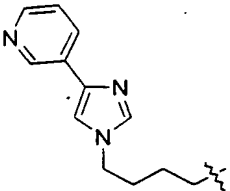
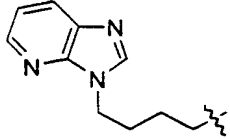
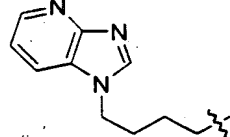
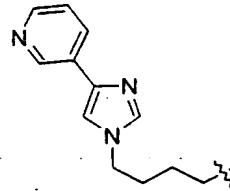
15 Minimum inhibitory concentrations (MICs) were determined by the NCCLS broth microdilution procedure for susceptibility testing for bacteria that grow aerobically (National Committee for Clinical Laboratory Standards, 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard. NCCLS Document M7-A4. National Committee for Clinical Laboratory Standards, Villanova, PA.). Stock solutions were prepared on the day of the test and appropriate aliquots were added to cation adjusted Mueller-Hinton broth (CAMHB) or *Haemophilus* test media. Two-fold serial dilutions were prepared and added to wells in microtiter plates. Final test concentrations ranged from 16 to 0.015 µg/ml. Broth cultures of bacteria inoculated from growth on overnight plates for all test bacteria except *Streptococcus pneumoniae* and *Haemophilus influenzae* were incubated at 35°C and then adjusted to the Kirby Bauer standard and diluted in CAMHB to achieve a final inoculum concentration of approximately 5x10⁵ CFU/ml. Inocula for *S. pneumoniae* and *H. influenzae* were prepared by directly suspending colonies from an overnight plate, adjusting the turbidity and diluting as above. *S. pneumoniae* media was supplemented with 2.5% lysed horse blood. All plates were incubated in ambient air at 35°C for 20-24 h for *S. pneumoniae* and *Haemophilus influenzae* and 16-20 h for all other bacteria.

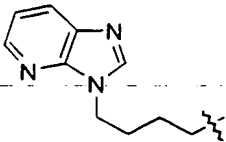
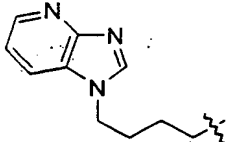
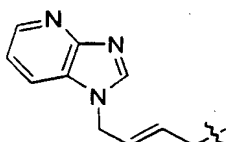
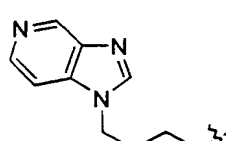
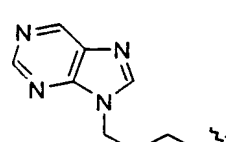
35 The MIC endpoints were determined by reading the lowest concentration of test compound that completely inhibited the growth of the test bacteria.

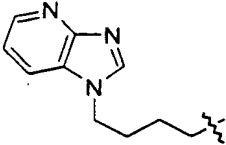
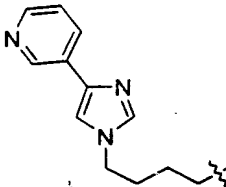
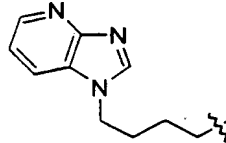
5 **Table 1. ¹H-NMR and Mass Spectral Data for Selected Compounds (I) of the Invention**



Compound	X	R ₆	R ₁₃	R	Spectroscopic Data
A	H	OCH ₃	CH ₃		M+H ⁺ = 782
B	H	OCH ₃	CH ₃		M+H ⁺ = 810
C	H	OCH ₃	CH ₃		¹ H-NMR (300 MHz, CHCl ₃) δ 8.43 (d, 1H), 8.15 (s, 1H), 8.08 (d, 1H), 7.17-7.14 (m, 1H), 5.20 (m, 1H), 4.42-4.10 (m, 4H), 3.8-3.43 (m, 4H), 3.38-3.0 (m, 4H), 2.98-2.86 (m, 2H), 2.62 (s, 3H), 2.57 (s, 6H), 2.1-1.10 (m, 31H)
D	H	OCH ₃	CH ₃		M+H ⁺ = 772

E	H	OCH ₃	CH=CH ₂		M+H ⁺ = 798
F	H	OCH ₃	CH=CH ₂		¹ H-NMR (300 MHz, CHCl ₃) δ 8.43 (d, 1H), 8.15 (s, 1H), 8.08 (d, 1H), 7.17-7.14 (m, 1H), 6.05-5.93 (m, 1H), 5.45-5.30 (m, 2H), 5.39 (s, 1H), 5.20 (s, 1H), 4.42-4.10 (m, 4H), 3.8-3.43 (m, 4H), 3.38-3.0 (m, 4H), 2.98-2.86 (m, 2H), 2.62 (s, 3H), 2.57 (s, 6H), 2.1-1.10 (m, 26H), 0.95 (d, 3H)
G	H	OCH ₃	CH=CH ₂		¹ H-NMR (300 MHz, CHCl ₃) δ 8.59 (d, 1H), 8.15 (s, 1H), 7.76 (d, 1H), 7.17-7.14 (m, 1H), 6.11-5.93 (m, 1H), 5.45-5.30 (m, 2H), 5.22 (s, 1H), 5.17 (s, 1H), 4.30-4.10 (m, 4H), 3.8-3.43 (m, 4H), 3.28-3.12 (m, 4H), 2.98-2.86 (m, 2H), 2.62 (s, 3H), 2.48-2.39 (m, 1H), 2.38 (s, 6H), 2.1-1.10 (m, 26H), 1.1 (d, 3H)
H	H	OCH ₃	CH ₂ CH ₂ CH ₃		M+H ⁺ = 826

J	H	OCH ₃	CH ₂ CH ₂ CH ₃		M+H ⁺ = 800
K	H	OCH ₃	CH ₂ CH ₂ CH ₃		M+H ⁺ = 800
L	H	OCH ₃	CH ₂ CH ₂ CH ₃		¹ H-NMR (300 MHz, CHCl ₃) δ 8.59 (d, 1H), 8.09 (s, 1H), 7.78 (d, 1H), 7.17-7.14 (m, 1H), 5.95-5.71 (m, 2H), 4.95 (d, 1H), 4.81 (d, 1H), 4.42-4.10 (m, 4H), 3.62-3.43 (m, 2H), 3.32-3.0 (m, 4H), 2.57 (s, 3H), 2.48-2.39 (m, 1H), 2.30 (s, 6H), 1.72-1.10 (m, 30H), 0.95 (d, 3H), 0.85 (t, 2H)
M	H	OCH ₃	CH ₂ CH ₂ CH ₃		¹ H-NMR (300 MHz, CHCl ₃) δ 9.20 (s, 1H), 8.43 (d, 1H), 8.02 (s, 1H), 7.41 (d, 1H), 5.15 (m, 1H), 4.42-4.10 (m, 4H), 3.82-3.42 (m, 5H), 3.32-3.02 (m, 4H), 2.57 (s, 3H), 2.48-2.39 (m, 1H), 2.30 (s, 6H), 2.10-0.95 (m, 39H)
N	H	OCH ₃	CH ₂ CH ₂ CH ₃		¹ H-NMR (300 MHz, CHCl ₃) δ 9.20 (s, 1H), 9.01 (s, 1H), 8.17 (s, 1H), 4.95 (d, 1H), 4.42-4.10 (m, 4H), 3.82-3.42 (m, 5H), 3.32-3.02 (m, 4H), 2.57 (s, 3H), 2.48-2.39 (m, 1H), 2.30 (s, 6H), 2.10-

					0.95 (m, 24H), 0.95 (d, 3H), 0.85 (t, 2H)
O	F	OCH ₃	CH ₂ CH ₂ CH ₃		¹³ C-NMR (75 MHz, CDCl ₃) δ 216.6, 202.7 (d, <i>J</i> = 28.0 Hz), 166.5 (d, <i>J</i> = 23.0 Hz), 157.3, 156.3, 145.0, 144.8, 126.0, 118.0, 118.0, 104.3, 97.8 (d, <i>J</i> = 204.9 Hz), 82.1, 80.7, 78.6, 70.4, 69.7, 65.8, 60.7, 53.5, 49.2, 45.0, 44.5, 42.4, 40.9, 40.2, 39.5, 39.2, 30.9, 28.2, 27.3, 25.2 (d, <i>J</i> = 22.3 Hz), 24.4, 21.2, 19.8, 19.1, 17.9, 15.0, 14.7, 13.7, 13.6. M+H ⁺ = 818
P	H	H	CH ₂ CH ₂ CH ₃		¹ H-NMR (300 MHz, CHCl ₃) δ 9.10 (s, 1H), 8.52 (s, 1H), 8.17 (d, 1H), 7.60 (s, 1H), 6.42 (s, 1H), 4.42-4.21 (m, 1H), 4.12-3.95 (m, 2H), 3.65-0.85 (m, 58H)
Q	H	H	CH ₂ CH ₂ CH ₃		M+H ⁺ = 770

5 Table 2. In Vitro Susceptibility (MIC in $\mu\text{g/ml}$)

Compound	Microorganism				
	<i>E. coli</i> OC2605	<i>S. aureus</i> ATCC29213	<i>E. faecalis</i> ATCC29212	<i>S. pneumoniae</i> ATCC49619	<i>H. influenzae</i> OC4883
Erythromycin	>16	0.5	1	0.06	1
A	>16	0.25	0.12	0.03	8
B	>16	0.5	0.25	0.03	16
C	>16	2	0.5	0.12	>16
D	>16	1	0.12	0.03	8
E	>16	0.5	0.12	0.03	8
F	>16	0.5	0.12	0.06	8
H	>16	1	0.25	0.06	8
J	>16	0.5	0.25	0.06	8
K	4	0.25	0.06	≤ 0.015	2
L	4	0.25	0.12	0.03	4
M	8	0.25	0.06	0.03	1
N	16	0.5	0.12	0.06	4
O	2	0.12	0.03	≤ 0.015	≤ 0.25
P	>16	>16	16	2	>16
Q	>16	8	1	0.25	>16
Compound of formula I wherein $R_6 = \text{O-3-(quinolin-3-}$	8	0.12	0.06	≤ 0.015	1

yl)prop-2-enyl, R ₁₃ = n-propyl, X = H, R = H					
Compound of formula I wherein R ₆ = O-3-(6-fluoro-quinolin-3-yl)prop-2-enyl, R ₁₃ = n-propyl, X = H, R = H	16	0.12	0.06	≤0.015	4
Compound of formula I wherein R ₆ = O-3-(6-chloro-quinolin-3-yl)prop-2-enyl, R ₁₃ = n-propyl, X = H, R = H	16	0.25	0.12	<u>0.03</u>	8

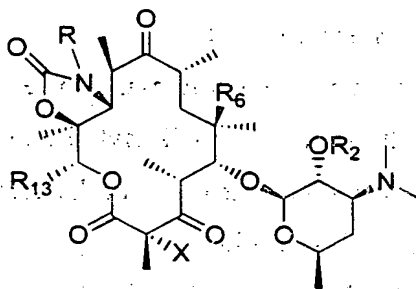
5

CLAIMS

We claim:

10

1. A compound of the formula I:



15

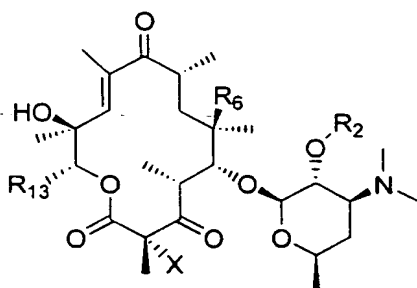
wherein:

20 X is H, F, Cl, Br, or I;

R₂ is selected from H, -COCH₃, and -COPhenyl;R₆ is selected from H and -O-R₄ where R₄ is substituted or unsubstituted alkyl (C₁-C₁₀), substituted or unsubstituted alkenyl (C₂-C₁₀), or substituted or unsubstituted alkynyl (C₂-C₁₀);

25 R₁₃ is selected from H, (C₁ - C₈) alkyl, 1-alkenyl (C₂-C₈), 1-alkynyl (C₂-C₈), substituted (C₁ - C₈)alkyl, and -CH₂-R" where R" is selected from H, (C₁ - C₈) alkyl, substituted (C₁ - C₈)alkyl, cycloalkyl, alkenyl (C₂- C₈), alkynyl (C₂-C₈), aryl, substituted-aryl, (C₁ - C₈) alkylaryl, heterocyclo, and substituted heterocyclo; provided that R₁₃ can not be ethyl;

30 R is selected from H, aryl, substituted-aryl, heterocyclo, substituted-heterocyclo, cycloalkyl, C₁-C₈-alkyl and C₁-C₈-alkenyl optionally substituted with one or more substituents selected from the group of aryl, substituted-aryl, heterocyclo, substituted-heterocyclo, hydroxy, C₁-C₆-alkoxy;



5

2

wherein;

10 X is H, F, Cl, Br, or I;

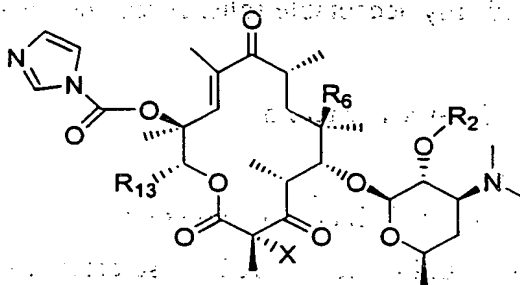
R₆ is selected from H and (C₁ - C₈) alkoxy;

R₁₃ is selected from H, (C₁ - C₈) alkyl, 1-alkenyl (C₂ - C₈), 1-alkynyl (C₂ - C₈), substituted (C₁ - C₈) alkyl, and -CH₂-R" where R" is selected from H, (C₁ - C₈) alkyl, substituted (C₁ - C₈) alkyl, cycloalkyl, alkenyl (C₂ - C₈), alkynyl (C₂ - C₈), aryl, substituted-aryl, (C₁ - C) alkylaryl, heterocyclo, and substituted heterocyclo;

15

provided that R₁₃ can not be ethyl.

5. A compound of the formula:



20

25 wherein:

X is H, F, Cl, Br, or I;

R₆ is selected from H and (C₁ - C₈) alkoxy;

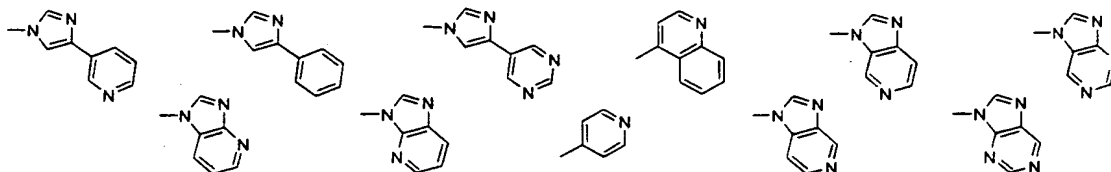
R₁₃ is selected from H, (C₁ - C₈) alkyl, 1-alkenyl (C₂ - C₈), 1-alkynyl (C₂ - C₈), substituted (C₁ - C₈) alkyl, and -CH₂-R" where R" is selected from H, (C₁ - C₈) alkyl, substituted (C₁ - C₈) alkyl, cycloalkyl, alkenyl (C₂ - C₈), alkynyl (C₂ - C₈), aryl, substituted-aryl, (C₁ - C₈) alkylaryl, heterocyclo, and substituted heterocyclo;

30

- 5 provided that R_{13} can not be ethyl; and
 R_2 is hydrogen or a hydroxyl protecting group.

6. The compound of claim 1 wherein substituted heterocyclo is selected from moieties of the formula:

10



- 15 7. The compound of claim 1 wherein :

X is H or F;

R_2 is as described in claim 1,

R_6 is $-O-R_a$ where R_a is substituted or unsubstituted alkyl (C_1-C_{10}), substituted or unsubstituted alkenyl (C_2-C_{10}), or substituted or unsubstituted alkynyl (C_2-C_{10});

- 20 R_{13} is selected from H, ($C_1 - C_8$) alkyl, 1-alkenyl (C_2-C_8), 1-alkynyl (C_2-C_8), haloalkyl, and $-CH_2-R''$ where R'' is selected from H, ($C_1 - C_8$) alkyl, haloalkyl, 1-alkenyl (C_2-C_8), 1-alkynyl (C_2-C_8), phenyl, and ($C_1 - C_8$) alkylphenyl;
 provided that R_{13} can not be ethyl;

R is H;

- 25 and the pharmaceutically acceptable salts, esters and pro-drug forms thereof.

8. The compound of claim 1 wherein:

X is H, F, Cl, Br, or I;

R_2 is selected from H, $-COCH_3$ and $-COPhenyl$;

- 30 R_6 is selected from H and $-O-R_a$ where R_a is selected from optionally substituted 3-(quinolin-3-yl)prop-2-enyl, optionally substituted 3-(quinolin-3-yl)prop-2-ynyl, optionally substituted 3-(quinolin-6-yl)prop-2-enyl, optionally substituted 3-(quinolin-6-yl)prop-2-ynyl, optionally substituted 3-(quinolin-7-yl)prop-2-enyl, optionally substituted 3-phenylprop-2-enyl, optionally substituted 3-(naphth-1-yl)prop-2-enyl,
 35 optionally substituted 3-(naphth-1-yl)prop-2-ynyl, optionally substituted 3-(naphth-2-yl)prop-2-enyl, optionally substituted 3-(naphth-2-yl)prop-2-ynyl, optionally substituted 5-phenylpent-4-en-2-ynyl, optionally substituted 3-(fur-2-yl)prop-2-ynyl,

- 5 optionally substituted 3-(thien-2-yl)prop-2-enyl, optionally substituted 3-(carbazol-3-yl)prop-2-enyl, and optionally substituted 3-(quinoxalin-6-yl)prop-2-enyl;
R₁₃ is selected from H, (C₁ - C₈) alkyl, 1-alkenyl (C₂-C₈), 1-alkynyl (C₂-C₈), substituted (C₁ - C₈)alkyl, and -CH₂-R" where R" is selected from H, (C₁ - C₈) alkyl, substituted (C₁ - C₈)alkyl, cycloalkyl, alkenyl (C₂- C₈), alkynyl (C₂-C₈), aryl,
10 substituted-aryl, (C₁ - C₈) alkylaryl, heterocyclo, and substituted heterocyclo;
provided that R₁₃ can not be ethyl;
R is selected from H, aryl, substituted-aryl, heterocyclo, substituted-heterocyclo, cycloalkyl, C₁-C₈-alkyl and C₁-C₈-alkenyl optionally substituted with one or more substituents selected from the group of aryl, substituted-aryl, heterocyclo, substituted-
15 heterocyclo, hydroxy, C₁-C₆-alkoxy;
and the pharmaceutically acceptable salts, esters and pro-drug forms thereof.
9. The Compound of claim 1 where X = H, R₆ = H, R₁₃ = methyl, R = quinolin-4-ylbutyl.
10. The Compound of claim 1 where X = F, R₆ = H, R₁₃ = methyl, R = quinolin-4-ylbutyl.
- 20 11. The Compound of claim 1 where X = H, R₆ = OCH₃, R₁₃ = methyl, R = quinolin-4-ylbutyl.
12. The Compound of claim 1 where X = F, R₆ = OCH₃, R₁₃ = methyl, R = quinolin-4-ylbutyl.
- 25 13. The Compound of claim 1 where X = H, R₆ = H, R₁₃ = methyl, R = 4-phenylimidazol-1-ylbutyl.
14. The Compound of claim 1 where X = F, R₆ = H, R₁₃ = methyl, R = 4-phenylimidazol-1-ylbutyl.
15. The Compound of claim 1 where X = H, R₆ = OCH₃, R₁₃ = methyl, R = 4-phenylimidazol-1-ylbutyl.
- 30 16. The Compound of claim 1 where X = F, R₆ = OCH₃, R₁₃ = methyl, R = 4-phenylimidazol-1-ylbutyl.
17. The Compound of claim 1 where X = H, R₆ = H, R₁₃ = methyl, R = 4-(pyridin-3-yl)imidazol-1-ylbutyl.
- 35 18. The Compound of claim 1 where X = F, R₆ = H, R₁₃ = methyl, R = 4-(pyridin-3-yl)imidazol-1-ylbutyl.

- 5 19. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{methyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$.
20. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{methyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$.
21. The Compound of claim 1 where $X = H$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = \text{pyridin-4-ylbutyl}$.
- 10 22. The Compound of claim 1 where $X = F$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = \text{pyridin-4-ylbutyl}$.
23. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{methyl}$, $R = \text{pyridin-4-ylbutyl}$.
- 15 24. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{methyl}$, $R = \text{pyridin-4-ylbutyl}$.
25. The Compound of claim 1 where $X = H$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = 3H\text{-imidazo[4,5-b]pyridin-3-ylbutyl}$.
26. The Compound of claim 1 where $X = F$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = 3H\text{-imidazo[4,5-b]pyridin-3-ylbutyl}$.
- 20 27. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{methyl}$, $R = 3H\text{-imidazo[4,5-b]pyridin-3-ylbutyl}$.
28. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{methyl}$, $R = 3H\text{-imidazo[4,5-b]pyridin-3-ylbutyl}$.
- 25 29. The Compound of claim 1 where $X = H$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = 1H\text{-imidazo[4,5-b]pyridin-1-ylbutyl}$.
30. The Compound of claim 1 where $X = F$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = 1H\text{-imidazo[4,5-b]pyridin-1-ylbutyl}$.
31. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{methyl}$, $R = 1H\text{-imidazo[4,5-b]pyridin-1-ylbutyl}$.
- 30 32. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{methyl}$, $R = 1H\text{-imidazo[4,5-b]pyridin-1-ylbutyl}$.
33. The Compound of claim 1 where $X = H$, $R_6 = H$, $R_{13} = n\text{-propyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$.
- 35 34. The Compound of claim 1 where $X = F$, $R_6 = H$, $R_{13} = n\text{-propyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$.

- 5 35. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 4$ -
(pyridin-3-yl)imidazol-1-ylbutyl.
36. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 4$ -
(pyridin-3-yl)imidazol-1-ylbutyl.
37. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 1H$ -
10 imidazo[4,5-c]pyridin-1-ylbutyl.
38. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 1H$ -
imidazo[4,5-c]pyridin-1-ylbutyl.
39. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 3H$ -
imidazo[4,5-c]pyridin-3-ylbutyl.
- 15 40. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 3H$ -
imidazo[4,5-c]pyridin-3-ylbutyl.
41. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R =$
purin-7-ylbutyl.
42. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R =$
20 purin-7-ylbutyl.
43. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R =$
purin-9-ylbutyl.
44. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R =$
purin-9-ylbutyl.
- 25 45. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 1H$ -
imidazo[4,5-b]pyridin-1-ylbut-2-enyl.
46. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 1H$ -
imidazo[4,5-b]pyridin-1-ylbut-2-enyl.
47. The Compound of claim 1 where $X = H$, $R_6 = H$, $R_{13} = n$ -propyl, $R = 4$ -
30 (pyrimidin-5-yl)imidazol-1-ylbutyl.
48. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = n$ -propyl, $R = 4$ -
(pyrimidin-5-yl)imidazol-1-ylbutyl.
49. The Compound of claim 1 where $X = H$, $R_6 = H$, $R_{13} = n$ -propyl, $R = 1H$ -
imidazo[4,5-b]pyridin-1-ylbutyl.
- 35 50. The Compound of claim 1 where $X = F$, $R_6 = H$, $R_{13} = n$ -propyl, $R = 1H$ -
imidazo[4,5-b]pyridin-1-ylbutyl.

- 5 51. The Compound of claim 1 where $X = H$, $R_6 = H$, $R_{13} = n\text{-propyl}$, $R = 3H\text{-imidazo}[4,5-b]\text{pyridin-3-ylbutyl}$.
52. The Compound of claim 1 where $X = F$, $R_6 = H$, $R_{13} = n\text{-propyl}$, $R = 3H\text{-imidazo}[4,5-b]\text{pyridin-3-ylbutyl}$.
53. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = n\text{-propyl}$, $R = 3H\text{-imidazo}[4,5-b]\text{pyridin-3-ylbutyl}$.
- 10 54. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = n\text{-propyl}$, $R = 3H\text{-imidazo}[4,5-b]\text{pyridin-3-ylbutyl}$.
55. The Compound of claim 1 where $X = H$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 4\text{-phenylimidazol-1-ylbutyl}$.
- 15 56. The Compound of claim 1 where $X = F$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 4\text{-phenylimidazol-1-ylbutyl}$.
57. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 4\text{-phenylimidazol-1-ylbutyl}$.
58. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 4\text{-phenylimidazol-1-ylbutyl}$.
- 20 59. The Compound of claim 1 where $X = H$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$.
60. The Compound of claim 1 where $X = F$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$.
- 25 61. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$.
62. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyridin-3-yl)imidazol-1-ylbutyl}$.
63. The Compound of claim 1 where $X = H$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyrimidin-5-yl)imidazol-1-ylbutyl}$.
- 30 64. The Compound of claim 1 where $X = F$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyrimidin-5-yl)imidazol-1-ylbutyl}$.
65. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyrimidin-5-yl)imidazol-1-ylbutyl}$.
- 35 66. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 4\text{-(pyrimidin-5-yl)imidazol-1-ylbutyl}$.

- 5 67. The Compound of claim 1 where $X = H$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 3H$ -imidazo[4,5-b]pyridin-3-ylbutyl.
68. The Compound of claim 1 where $X = F$, $R_6 = H$, $R_{13} = \text{vinyl}$, $R = 3H$ -imidazo[4,5-b]pyridin-3-ylbutyl.
69. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 3H$ -imidazo[4,5-b]pyridin-3-ylbutyl.
- 10 70. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{vinyl}$, $R = 3H$ -imidazo[4,5-b]pyridin-3-ylbutyl.
71. The Compound of claim 1 where $X = H$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = 1H$ -imidazo[4,5-b]pyridin-1-ylbutyl.
- 15 72. The Compound of formula I where $X = F$, $R_6 = H$, $R_{13} = \text{methyl}$, $R = 1H$ -imidazo[4,5-b]pyridin-1-ylbutyl.
73. The Compound of claim 1 where $X = H$, $R_6 = OCH_3$, $R_{13} = \text{methyl}$, $R = 1H$ -imidazo[4,5-b]pyridin-1-ylbutyl.
74. The Compound of claim 1 where $X = F$, $R_6 = OCH_3$, $R_{13} = \text{methyl}$, $R = 1H$ -imidazo[4,5-b]pyridin-1-ylbutyl.
- 20 75. A pharmaceutical composition for treating bacterial infections comprising an effective amount of a compound selected from claim 1 in association with a pharmaceutically acceptable carrier.
76. A method of treating bacterial infections in mammals by administering to
25 such mammal suffering from such infection a therapeutically acceptable amount of a compound selected from claim 1.

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
26 October 2000 (26.10.2000)

PCT

(10) International Publication Number
WO 00/62783 A3

(51) International Patent Classification: C07H 17/08,
A61K 31/70

(74) Agents: CIAMPORCERO, Audley et al.; Johnson &
Johnson, One Johnson & Johnson Plaza, New Brunswick,
NJ 08933-7003 (US).

(21) International Application Number: PCT/US00/10276

(22) International Filing Date: 13 April 2000 (13.04.2000)

(81) Designated States (national): AE, AL, AM, AT, AU, AZ,
BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK,
DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL,
IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,
LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT,
RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,
UG, UZ, VN, YU, ZA, ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/129,729 16 April 1999 (16.04.1999) US
06/131,383 28 April 1999 (28.04.1999) US
60/140,175 18 June 1999 (18.06.1999) US
60/172,159 17 December 1999 (17.12.1999) US
60/172,154 17 December 1999 (17.12.1999) US

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent
(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent
(AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU,
MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GW, ML, MR, NE, SN, TD, TG).

(71) Applicants: ORTHO-MCNEIL PHARMACEUTI-
CAL, INC. [US/US]; US Route 202, Raritan, NJ 08869
(US); KOSAN BIOSCIENCES, INC. [US/US]; 3832
Bay Center Place, Hayward, CA 94545 (US).

Published:

With international search report.

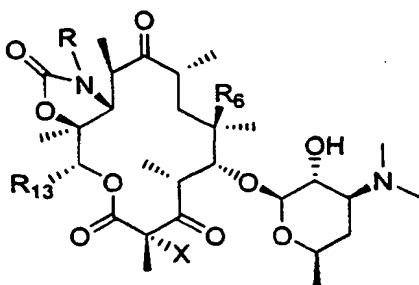
(72) Inventors: HLASTA, Dennis; 5008 Davis Drive,
Doylestown, PA 18901 (US); HENNINGER, Todd; 125
Bermuda Dr., Neshanic Station, NJ 08853 (US); GRANT,
Eugene; 1011 Edison Glen Terrace, Edison, NJ 08837
(US); KHOSLA, Chaitin; 740 Lapara Ave., Palo Alto,
CA 94306 (US); CHU, Daniel; 3767 Benton Street, Santa
Clara, CA 95051 (US).

(88) Date of publication of the international search report:
15 February 2001

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: KETOLIDE ANTIBACTERIALS

WO 00/62783 A3



(a)

(57) Abstract: A series of ketolide antibiotics in the macrolide family of formula (a), intermediates used in their manufacture and pharmaceutical compositions containing them. The compounds are erythromycin analogues useful in the treatment of bacterial and protozoal infections and in the treatment of other conditions involving gastric motility.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/10276

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07H17/08 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07H A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2 754 821 A (HOECHST MARION ROUSSEL INC) 24 April 1998 (1998-04-24) abstract page 1, structure I page 8, structure VI page 10, structure IX	1,4-6, 75,76
Y	US 5 866 549 A (CHU DANIEL T ET AL) 2 February 1999 (1999-02-02) abstract column 2, structure III column 75, scheme 2, structure 11 column 77, scheme 3b, structure 12 column 3, structure V --- -/--	1-8,75, 76

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

8 November 2000

Date of mailing of the international search report

13. 11. 2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

de Nooy, A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/10276

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 49315 A (KOSAN BIOSCIENCES INC ;UNIV LELAND STANFORD JUNIOR (US)) 5 November 1998 (1998-11-05) figure 9, structure 95 figure 12b, structures 318-320 ---	1-8,75, 76
Y	US 5 635 485 A (AGOURIDAS CONSTANTIN ET AL) 3 June 1997 (1997-06-03) cited in the application abstract column 1, structure I column 7, structure II ---	1-8,75, 76
Y	WO 99 03986 A (KOSAN BIOSCIENCES INC) 28 January 1999 (1999-01-28) cited in the application figure 3 ---	1-8,75, 76
P,X	WO 99 35157 A (WU YONG JIN ;PFIZER PROD INC (US)) 15 July 1999 (1999-07-15) the whole document -----	1-8,75, 76

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/10276

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 76 is directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

As a result of the prior review under R. 40.2(e) PCT,
no additional fees are to be refunded.

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☒ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-8 (in part), 75 (in part), 76 (in part)

Compounds of formulae 1, 2 or 3 of claims 1, 4 or 5, respectively (see also page 3 and 8 of the description), pharmaceutical compositions and uses of these compounds, where R13 is H.

2. Claims: 1-8 (in part), 9-74 (in full), 75 (in part), 76 (in part)

Compounds of formulae 1, 2 or 3 of claims 1, 4 or 5, respectively (see also page 3 and 8 of the description), pharmaceutical compositions and uses of these compounds, where R13 is (C1-C8) alkyl, 1-alkenyl (C2-C8) or 1-alkynyl (C2-C8) provided R13 can not be ethyl.

3. Claims: 1-8 (in part), 75 (in part), 76 (in part)

Compounds of formulae 1, 2 or 3 of claims 1, 4 or 5, respectively (see also page 3 and 8 of the description), pharmaceutical compositions and uses of these compounds, where R13 is substituted (C1-C8) alkyl.

4. Claims: 1-8 (in part), 75 (in part), 76 (in part)

Compounds of formulae 1, 2 or 3 of claims 1, 4 or 5, respectively (see also page 3 and 8 of the description), pharmaceutical compositions and uses of these compounds, where R13 is -CH₂-R" where R" is selected from H, (C1-C8) alkyl, substituted (C1-C8) alkyl, cycloalkyl, alkenyl (C2-C8), alkynyl (C2-C8), aryl, substituted aryl, (C1-C8) alkylaryl, heterocyclo and substituted heterocyclo provided R13 can not be ethyl. Furthermore, R13 is not any of the substituents of subject 2 or 3.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 00/10276

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
FR 2754821	A	24-04-1998	NONE	
US 5866549	A	02-02-1999	AU 4178097 A	26-03-1998
			BG 103292 A	30-12-1999
			BR 9711661 A	24-08-1999
			CN 1237183 A	01-12-1999
			CZ 9900685 A	11-08-1999
			EP 0929563 A	21-07-1999
			HR 970473 A	31-08-1998
			NO 991022 A	03-05-1999
			PL 332009 A	16-08-1999
			SI 20023 A	29-02-2000
			WO 9809978 A	12-03-1998
			US 6075133 A	13-06-2000
			US 6028181 A	22-02-2000
WO 9849315	A	05-11-1998	AU 7172298 A	24-11-1998
			EP 0979286 A	16-02-2000
			US 6117659 A	12-09-2000
US 5635485	A	03-06-1997	FR 2719587 A	10-11-1995
			AP 558 A	07-11-1996
			AT 172203 T	15-10-1998
			AU 684027 B	27-11-1997
			AU 2449995 A	29-11-1995
			BG 100951 A	29-08-1997
			BR 9507700 A	19-08-1997
			CA 2189271 A	09-11-1995
			CN 1151746 A,B	11-06-1997
			CN 1229082 A	22-09-1999
			CZ 9603214 A	13-08-1997
			DE 69505296 D	19-11-1998
			DE 69505296 T	22-04-1999
			DK 680967 T	23-06-1999
			EP 0680967 A	08-11-1995
			ES 2122472 T	16-12-1998
			FI 964395 A	31-10-1996
			WO 9529929 A	09-11-1995
			HU 75698 A	28-05-1997
			JP 2992540 B	20-12-1999
			JP 8053489 A	27-02-1996
			JP 11152296 A	08-06-1999
			LV 11739 A	20-04-1997
			LV 11739 B	20-08-1997
			MD 1233 B	31-05-1999
			NO 964654 A	04-11-1996
			NZ 285416 A	27-07-1997
			PL 317071 A	03-03-1997
			RO 113350 B	30-06-1998
			SI 680967 T	28-02-1999
			SK 140296 A	08-04-1998
			US 6100404 A	08-08-2000
			ZA 9503501 A	02-05-1996
WO 9903986	A	28-01-1999	US 6066721 A	23-05-2000
			AU 8412398 A	10-02-1999
			CN 1267332 T	20-09-2000
			EP 0996714 A	03-05-2000

Information on patent family members

PCT/US 00/10276

Form PCT/ISA/210 (patent family annex) (July 1992)

THE UNITED STATES OF AMERICA
DEPARTMENT OF JUSTICE
WASHINGTON, D. C. 20535

TO: THE ATTORNEY GENERAL
FROM: THE ATTORNEY GENERAL
SUBJECT: [Illegible]

RE: [Illegible]

[Illegible text block]

[Illegible text block]

[Illegible text block]